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Alterations of β-adrenoceptor responsiveness in postischemic myocardium after 72 h of reperfusion

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Abstract

To determine the effect of a completely developed reperfused myocardial infarction model on β-adrenoceptor responsiveness, we induced a 90-min regional ischemia followed by 72 h of reperfusion in dog hearts. Regional myocardial blood flow was determined after 60 min of ischemia using radioactive microspheres. β-adrenoceptor density was reduced in the ischemic endocardium (95 ± 16 fmol/mg) and epicardium (160 ± 13 fmol/mg) compared to the nonischemic region (304 ± 21 fmol/mg). β-adrenoceptor density in the ischemic endocardium varied with the degree of collateral blood flow measured ($r^2 = 0.79$, $P < 0.05$); this relation was the opposite of that in the ischemic epicardium ($r^2 = 0.77$, $P < 0.05$). Higher levels of tissue catecholamines and G protein-coupled receptor kinase 2 (GRK2) were observed in the ischemic epicardium as compared to nonischemic tissue. Forskolin-induced adenylyl cyclase activities were depressed in both ischemic regions as compared to nonischemic region, correlating with a reduction in regional myocardial blood flow. Using forskolin stimulation as covariate, no difference in isoproterenol-induced adenylyl cyclase activity was identified in the different regions.

It is concluded that cAMP production induced by β-adrenoceptor activation is dependent upon adenylyl cyclase enzyme activity rather than β-adrenoceptor density in the ischemic myocardium. However, the density of the β-adrenoceptor in the viable ischemic regions can be modified by the presence of GRK2 and tissue catecholamines, an index of regional sympathetic efferent postganglionic nerve terminal activity.

Keywords: β-adrenoceptor; Adenylyl cyclase; Ischemia; Reperfusion; Catecholamine; Regional myocardial blood flow

1. Introduction

The β-adrenergic system plays an important role in the adaptation of the cardiovascular system to pathologic conditions. Activation of β-adrenoceptors by endogenous catecholamines modulates heart rate and cardiac contractility as well as vascular tone (Brodde, 1991). It has been reported that during ischemia, myocardial norepinephrine content increases and thus stimulates cardiac β-adrenoceptors (Strasser et al., 1990). This sustained β-adrenoceptor stimulation may induce uncoupling of the receptor from the G protein to ultimately reduce total β-adrenoceptor density (Brouri et al., 2002).

To prevent further damage to the ischemic myocardium, reperfusion of the jeopardized tissue has been performed with different techniques using pharmacological agents, angioplasty or surgical procedures. It is has been shown that reperfusion can be beneficial to the myocardium, even if this procedure is performed some time after the infarction (Hochman and Choo, 1987; Lavallée et al., 1983). However, changes in coronary artery perfusion can modulate sympathetic efferent neural tone to the heart and circulating catecholamine levels both of which can affect cardiac β-adrenoceptor function (Vatner et al., 1993).

Many studies have examined the effects of ischemia on cardiomyocyte β-adrenoceptors. However, relatively few have focused on the effect of completely developed reperfused myocardial infarction cardiac β-adrenoceptor function. Therefore, we designed the present study to characterize the changes in the ventricular β-adrenergic system in a protocol consisting of 90 min of regional ischemia followed by 72 h of

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reperfusion. We chose 72 h of reperfusion to ensure that myocardial infarction was fully developed without resulting in heart failure. Regional myocardial blood flow was measured at 60 min of ischemia to verify the correlation between intensity of the ischemia and changes observed in the β-adrenoceptror signaling pathways as well as ventricular catecholaminergic nerve terminals.

2. Materials and methods

2.1. Ischemia and reperfusion

Studies adhered to the guidelines on the care and uses of laboratory animals issued by the Canadian Council on Animal Care and were approved by a local committee. All surgical interventions were performed under sterile conditions.

Farm-bred Walker dogs of either sex, weighing 20–30 kg, were anesthetized with sodium pentobarbital (30 mg/kg, i.v.), intubated, and ventilated mechanically with room air. A lead II electrocardiogram was monitored continuously including during the first 6 h of reperfusion. After administering pancuronium bromide (0.1 mg/kg, i.v.), a left thoracotomy was performed at the fifth intercostal space. The heart was exposed and suspended in a pericardial cradle. Catheters were introduced into the left ventricular chamber through the left atrial appendage for pressure monitoring and into the left atrium for microsphere injection and blood sampling.

The left anterior descending coronary artery was isolated distal to its first branch and an electromagnetic flow meter was placed around it to monitor coronary blood flow. The left anterior descending coronary artery was then occluded for 90 min, followed by a 72 h reperfusion period. After 5 h of reperfusion, the chest was closed and the animals were returned to the kennel to recuperate after intramuscular administration of 3 mg/kg of gentamycin (Schering Canada), 2 million IU of penicillin (Penlong XL) and 0.5 mg of buprenorphine (Buprenex, Reckitt Beckinser, England). Additional doses of 0.5 mg buprenorphine, i.m., were given the next morning and every 12 h thereafter as needed. Gentamycin (3 mg/kg, i.m.) was administered to the animal each day.

To obtain basal values, three anesthetized dogs (sham) were submitted to thoracotomy, and the heart has been excised and used for the determination of the β-adrenoceptor density and adenylyl cyclase activity.

2.2. Measurement of regional and collateral myocardial blood flow

Regional myocardial blood flow was measured, as previously described (Rousseau et al., 1993). Briefly, after 60 min of occlusion, approximately 2–3 million microspheres [15-μm microspheres labeled with ^{51}Cr or ^{46}Sc (NEN Canada), adequately dispersed in a 1 ml suspending medium containing 0.01% Tween-80] were injected into the left atrial cavity over 20 s and flushed thereafter with 20 ml of saline. A reference arterial blood flow sample was collected from a femoral artery at that time via a 16-G butterfly needle (Abbott Laboratories) to avoid catheterization of the artery; hemostasis was insured by compression of the vessel for 15 min. Blood samples were obtained for 150 s, at a constant rate starting 15 s before microspheres were injected. Follow completion of the experiments, the four central slices of the left ventricle were divided into nonischemic and ischemic regions for regional myocardial blood flow analysis. The nonischemic area was selected from the wall opposite to the area at risk in the circumflex coronary artery territory. Sections from the central ischemic and nonischemic reference zones were weighed and counted in a gamma counter. Regional myocardial blood flow was expressed in ml/min/g of tissue and calculated according to the following equation: $Q_m = Q_r \times C_m/C_r$, where Q_m = myocardial blood flow (ml/min), Q_r = reference blood flow (ml/min), C_m = counts/min in the myocardial sample, and C_r = counts/min in the reference sample.

2.3. Infarct size

After 72 h of reperfusion, the animals were reanesthetized and the thoracic cavity was reopened. Just before sacrificing the animals, sodium-heparin (10,000 U, i.v.) was administered. The animals were then sacrificed by cardiac fibrillation. The heart was rapidly excised and infarct size quantified, as reported previously (Rousseau et al., 1993). The site of the left anterior descending coronary artery occlusion and the aorta above the ostia were cannulated and perfused with Evans blue dye (0.5%), and the left anterior descending coronary artery with saline (0.9%) at a constant pressure (100 mm Hg) for 5 min. The left ventricle was embedded in polyurethane foam (a gift of MIA Chemicals, Canada Fiberglass) and cut with a commercial meat slicer into 7-mm-thick transverse slices. The third slice was cut into two 3.5-mm-thick slices: one slice was rapidly frozen at −80 °C for subsequent analysis of tissue adenylyl cyclase activity and β-adrenoceptor determination. The other slices were immersed in 2,3,5-triphenyltetrazolium chloride (TTC 1.5% in Tris buffer 2.4%, pH 7.8) at 37 °C for 5 min. The normally perfused zone (Evans blue-positive), the area at risk (Evans blue-negative), and the infarct area (TTC and Evans blue-negative) were estimated by computer planimetry for each slice.

2.4. Membrane preparation

Tissue samples from ischemic endocardium (corresponding to necrotic tissue) and epicardium (corresponding to viable postischemic tissue) or from normally perfused (nonischemic) tissue were lysed with a polytron homogenizer (four bursts of 15 s at maximun speed) in 10 ml of ice-

cold buffer containing 5 mM Tris–HCl, pH 7.4, 2 mM EDTA, and protease inhibitors (5 μg/ml leupeptin, 5 μg/ml soybean trypsin inhibitor, and 10 μg/ml benzamidine). Lysates were centrifuged at 1000 × *g* for 5 min, and the supernatant were centrifuged at 45,000 × *g* for 20 min at 4 °C. The pelleted membranes were washed twice in the same buffer, resuspended in 0.5 ml of buffer containing 75 mM Tris–HCl, pH 7.4, 5 mM $MgCl_2$, 2 mM EDTA, protease inhibitors and immediately utilized for the adenylyl cyclase activity determination and radioligand binding assays described below. The supernatant of the first centrifugation at 45,000 × *g* was kept and used for G protein-coupled receptor kinase 2 (GRK2) determination. Protein content was determined according to the method of Bradford (Bio-Rad, Hercules, CA).

2.5. Adenylyl cyclase assay

Adenylyl cyclase activity was measured according to the method of Salomon et al. (1974). Briefly, 10 μg of membrane proteins was added to a total volume of 50 μl of a reaction mixture containing 120 μM ATP, 0.5 μCi [α-^{32}P]ATP, 100 μM cAMP, 53 μM GTP, 2.8 mM phosphoenolpyruvate, 0.2 U of pyruvate kinase, 1 U of myokinase, 30 mM Tris–HCl (pH 7.4), 2 mM $MgCl_2$, 0.8 mM EDTA, and 0.1 mM isobutylmethylxanthine. The final free Mg^{2+} concentration in the assay was 1.1 mM as calculated by the method of Iyengar and Birnbaumer (1982). Enzyme activity was determined in duplicate in the absence (basal activity) or in the presence of activators (isoproterenol 1 nM–100 μM or forskolin 100 μM).

2.6. Radioligand binding assay

Radioligand binding assays were conducted essentially as described previously (Rousseau et al., 1997), with 5 μg of membrane proteins in a total volume of 0.5 ml. Saturation experiments were carried out using 250 pM [^{125}ICYP, 75 mM Tris–HCl pH 7.4, 5 mM $MgCl_2$, and 2 mM EDTA in the presence or absence of 10 μM alprenolol to define nonspecific binding. The binding reactions were incubated at room temperature for 90 min and terminated by rapid filtration over Whatman GF/C glass fiber filters preincubated in a buffer containing 25 mM Tris–HCl, pH 7.4, 0.1% bovine serum albumin, and 0.3% polyethylenimine. The filtered membranes were then washed thrice with ice-cold 25 mM Tris–HCl, pH 7.4 and counted in a gamma-counter.

2.7. Western blotting of cytosolic G protein-coupled receptor kinase 2 (GRK2)

Protein content of the supernatant from membrane preparation was determined according to the method of Lowry (Bio-Rad). The proteins were separated on a sodium dodecyl sulfate-polyacrylamide gel electophoresis using a 10% slab gel. The proteins were then transferred electrophoretically to a nitrocellulose membrane which was blocked with 5% fat-free milk, 0.05% (v/v) Tween 20 in Tris-buffered saline (TBS, pH 7.5). After three washes with TBS-Tween 20 (0.05% v/v), membranes were incubated with anti-GRK2 (gift of Dr. Jeffrey L. Benovic, Thomas Jefferson University) at a dilution of 1:1000 overnight at 4 °C, followed by five washes with TBS-Tween 20. The immunoreactivity was finally revealed using a horseradish peroxidase-conjugated anti-mouse antibody (1:5000) and revealed by enhanced chemiluminescence (Renaissance, Mandel, Canada). Images were analysed using Kodak 1-D image Analysis software (version 3.5.5).

2.8. Glyoxylic acid-induced histofluorescence for catecholamines

Histofluorescence specific for catecholamines was performed as previously described (Himura et al., 1993). Tissue sections from fresh hearts were rapidly frozen on dry ice and stored at −80 °C until used. Blocks were mounted on a cryostat and cut into 7-μm sections. Sections were picked up on glass slides, dipped in sucrose–phosphate–glyoxylic acid (SPG) solution for 3 s according to the solution of de la Torre (de la Torre, 1980), dried and heated at 95 °C for 2.5 min, and viewed using a Leica epi-illumination microscope. All sections were photographed at the same magnification (400 ×) using a Nikon coolpix 995 camera. The color of the stained catecholamine profiles was then changed to red and other tissue sections to green. Number of pixels was then calculated using internal software and red pixels (catecholamines) were reported compared to red plus green pixels (total tissue).

2.9. Statistical analysis

Results are expressed as mean ± S.E.M. Hemodynamic data were analysed with a one-way analysis of variance. Comparison between the different regions was performed by analysis of variance followed by a Dunnett's analysis. Correlation between variables was estimated by linear regression analysis (Primer of Biostatistics version 3.0). Analysis of covariance was adapted for factorial experimental design and orthogonalization was made according to Winer (1971). Differences were considered statistically significant when $P < 0.05$.

3. Results

3.1. Hematologic and hemodynamics data

Thirteen dogs were included in this study. Hematologic and hemodynamic data are summarized in Table 1. Heart rate was similar throughout the entire time course of the experiments. Cardiac work, estimated by pressure rate product, was reduced significantly at the end of the ischemic

Table 1
Hematologic and hemodynamic data

Parameters	
Hematologic data (before anesthesia)	
Weight (kg)	22.1 ± 0.7
Hematocrit (%)	50 ± 3
Hemoglobin (g/100 ml)	17.0 ± 0.9
Platelets (10^9/l)	238 ± 17
White blood cells (10^9/l)	11.1 ± 0.7
Rectal temperature at reperfusion (°C)	38.1 ± 0.3
Hemodynamic data (Before occlusion)	
Heart rate	193.4 ± 7.6
BP	144.6 ± 4.6
PRP	280.1 ± 14.2
LAD flow	27.5 ± 3.9
After 90 min of occlusion	
Heart rate	191.5 ± 5.6
BP	130.9 ± 4.9[a]
PRP	248.6 ± 11.9[a]
After 3 h of reperfusion	
Heart rate	185.4 ± 4.7
BP	140.9 ± 5.8[a]
PRP	262.3 ± 14.3[a]
LAD flow	18.9 ± 1.8[a]
After 5 h of reperfusion	
Heart rate	197.9 ± 6.7
BP	132.0 ± 5.8[a]
PRP	262.4 ± 17.0[a]
LAD flow	17.7 ± 2.4[a]

Values are means ± S.E.M. BP, peak left ventricular systolic pressure (mm Hg); HR, heart rate (beats/min); LAD flow, left anterior descending coronary artery flow (ml/min); PRP, pressure-rate product (HR × BP/100).
[a] $P < 0.05$ vs. before occlusion.

period and during the reperfusion period. This reduction is mainly due to a reduction in the left ventricular systolic pressure. Left anterior descending coronary artery blood flow was also reduced during the reperfusion period as compared to basal conditions. These data indicate, as expected, that the development of myocardial infarction affects left ventricular function.

3.2. Infarct size

Area at risk, infarct size and collateral blood flow are reported in Table 2. The area at risk involved approximately 40% of the left ventricle. Infarct size, as a percentage of the area at risk, was 38.0 ± 5.0%. Fig. 1 shows the correlation ($r^2 = 0.67$, $P < 0.001$) between infarct size and transmural collateral blood flow after 60 min of occlusion. As predicted, infarct size is inversely correlated with transmural collateral blood flow.

Table 2
Area at risk, infarct size and transmural collateral blood flow

Infarct size	
Area at risk (% of left ventricle)	41.6 ± 1.7
Infarct (% of area at risk)	38.0 ± 5.0
Infarct (% of left ventricle)	16.0 ± 2.4
Transmural myocardial blood flow (ml/min/g)	
Ischemic area	
Endocardium	0.047 ± 0.021[a]
Epicardium	0.263 ± 0.054[a]
Nonischemic area	1.577 ± 0.120

[a] $P < 0.05$ vs. nonischemic area.

3.3. β-adrenoceptors density

β-adrenoceptor densities were determined in different regions of the left ventricle. The total β-adrenoceptor density was 304 ± 21 fmol/mg in the nonischemic region, being similar to β-adrenoceptors density in sham-operated dogs (301 ± 33 fmol/mg). β-adrenoceptor density in nonischemic tissue was significantly higher than that observed in the ischemic endocardium (95 ± 16 fmol/mg) or epicardium (160 ± 13 fmol/mg).

Analysis of covariance indicated that the relation between the β-adrenoceptor density in the ischemic regions and the regional blood flow during the ischemia was heterogeneous. In the ischemic endocardium, the relation was positive ($y = 363.8x + 75.1$, $r^2 = 0.62$, $P < 0.05$). That is with greater regional blood flow was associated with higher β-adrenoceptors density. On the other hand, in the ischemic epicardium, the relation is inverted ($y = -136.0x + 188.9$, $r^2 = 0.60$, $P < 0.05$). Namely, higher regional blood flow resulted in a reduction of β-adrenoceptor density.

3.4. Adenylyl cyclase activity

Fig. 2A illustrates the classical concentration-dependent increase in adenylyl cyclase activity measured in membrane preparations derived from nonischemic tissue that were stimulated with the nonselective β-adrenoceptor agonist isoproterenol. This result is similar to what was obtained in sham-operated dogs (basal value was 42.5 ± 3.5 pmol/min/mg of protein and maximum value was 71.8 ± 12.6 pmol/min/mg of protein). The response observed in the ischemic endocardium or epicardium demonstrates a significant reduction in both basal and maximal activity induced by isoproterenol as compared to nonischemic tissue ($P < 0.05$). As expected, the ischemic endocardium is

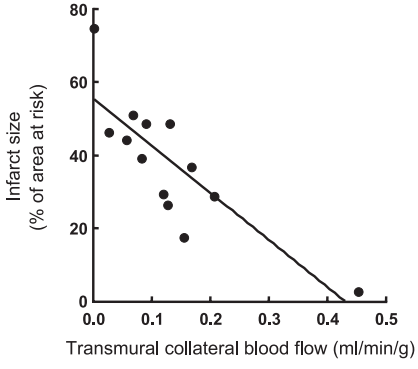

Fig. 1. Correlation between transmural collateral blood flow and infarct size expressed as percent of area at risk.

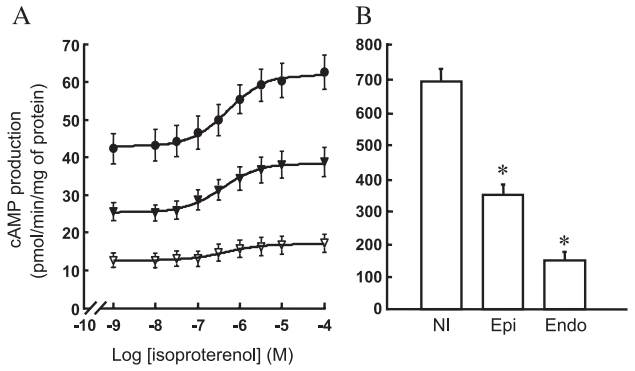

Fig. 2. (A) Adenylyl cyclase activity stimulated by nonselective β-adrenergic agonist isoproterenol in nonischemic (●), ischemic epicardium (▼) and ischemic endocardium (▽) regions ($n=13$). (B) Adenylyl cyclase activity stimulated by forskolin (100 μM) in nonischemic (NI), ischemic epicardium (Epi) or ischemic endocardium (Endo) regions. *$P<0.05$ vs. nonischemic region ($n=13$).

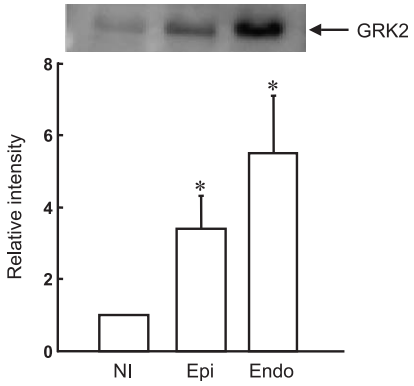

Fig. 4. G protein-coupled receptor kinase 2 (GRK2) levels in cytosolic fractions purified from ischemic epicardium (Epi), ischemic endocardium (Endo) and nonischemic region (NI). The autoradiogram shown is representative of $n=6$ independent experiments. *$P<0.05$ vs. nonischemic region.

affected to a greater extent than the epicardium. No difference was observed in the EC_{50} for the three regions (nonischemic 537 ± 112 nM; ischemic endocardium 559 ± 101 nM; ischemic epicardium 585 ± 175 nM; sham being 651 ± 47 nM).

Forskolin-stimulated adenylyl cyclase activity was also reduced in the ischemic region as compared to the nonischemic region (Fig. 2B). Ischemic endocardium region displayed a level of forskolin-induced adenylyl cyclase activity that was reduced by more than five times, and ischemic epicardium reduced by more than two times as compared to nonischemic region. Before the ischemia, the value for the forskolin was 689.4 ± 43 pmol/min/mg of protein is similar to the nonischemic region value.

Analysis of covariance between the isoproterenol-induced and forskolin-induced adenylyl cyclase activity indicate that both activities were closely related. The adjusted mean of isoproterenol-induced adenylyl cyclase activation normalized to forskolin-induced adenylyl cyclase activity as a covariate were 40.4 ± 3.4 pmol/min/mg of protein for the nonischemic region, 36.9 ± 1.4 pmol/min/mg of protein for the ischemic endocardium, and 43.6 ± 2.5 pmol/min/mg of protein for the ischemic epicardium. Thus, no significant difference was observed between regions.

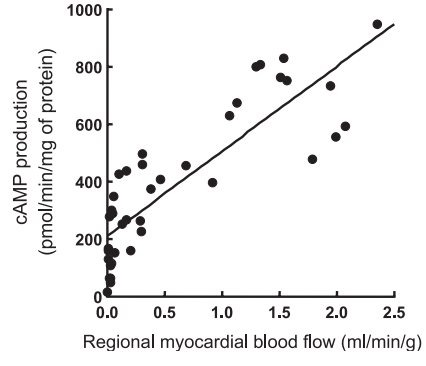

Fig. 3. Correlation between myocardial blood flow measured at 60 min of ischemia and adenylyl cyclase activity stimulated by forskolin (100 μM). $r^2=0.72$; $P<0.01$.

Interestingly, there is a strong correlation between the myocardial regional blood flow at 60 min and the activity of adenylyl cyclase after 72 h of reperfusion generated by forskolin, as indicated in Fig. 3 ($r^2=0.72$; $P<0.01$). This indicates that the adenylyl cyclase activity observed after 72 h of reperfusion was closely related to regional myocardial blood flow measured after 60 min of ischemia.

3.5. Cardiac GRK2 content

In order to test if loss of β-adrenoceptor responsiveness can be related to changes in GRK2 levels, we determined their expression in the different ventricular regions by Western blot analysis. As observed in Fig. 4, expression of GRK2 was significantly increased in the different ischemic regions (endocardium and epicardium) as compared to the nonischemic region ($P<0.05$).

3.6. SPG histofluororescence

Catecholamine content evaluated by tissue SPG histofluorescence indicate that the level of catecholamines increased significantly in the ischemic epicardium (2.49±0.26) as compared to nonischemic region (1.43±0.18) or ischemic endocardial tissue (0.89 ± 0.8; Fig. 5).

4. Discussion

In the present study, we report that after 90 min of ischemia followed by 72 h of reperfusion in a canine model of reperfused myocardial infarction, the β-adrenoceptor signaling pathway is altered in the postischemic myocardium. These alterations include a reduction of the β-adrenoceptor density and adenylyl cyclase activity in association

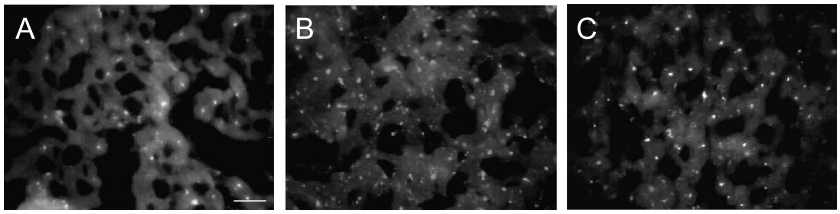

Fig. 5. Left ventricle SPG-induced histofluorescence demonstrating catecholaminergic profiles in ischemic endocardium (A) and epicardium (B) or in nonischemic region (C). Horizontal bar, 50 μm.

with an increase in the tissue catecholamine content and GRK2 protein content.

Reduction of β-adrenoceptor density correlates with the intensity of the regional myocardium blood flow measured after 60 min of ischemia and thus myocardial damage. It has been reported previously that after 60 min of ischemia and 15 to 20 min of reperfusion, β-adrenoceptor density in the ischemic endocardium is slightly reduced (Karliner et al., 1989; Vatner et al., 1993). Our data indicate that this reduction is greater following longer periods of reperfusion presumably because cells expressing β-adrenoceptors such as cardiomyocytes are progressively replaced by scar tissue.

For the ischemic epicardium, previous studies have reported that following 60 min of ischemia and 15 to 20 min of reperfusion, β-adrenoceptor density remains unchanged compared to nonischemic tissue (Karliner et al., 1989; Vatner et al., 1993). After 72 h of reperfusion, we observed that the number of β-adrenoceptors in the ischemic epicardium was reduced by about 50% compared to the nonischemic region. Interestingly, we observe that this reduction in β-adrenoceptor density is inversely correlated with the regional myocardial blood flow as opposed to what it occurs in the ischemic endocardium. These results suggest that in the ischemic epicardium, the β-adrenoceptors are subjects to mechanisms leading to downregulation which are not observed in the ischemic endocardium. Classically, downregulation of receptors is due to sustained activation. Evaluation of regional catecholamine content by the sensitive glyoxylic acid-induced histofluorescence (Himura et al., 1993) indicated a significant elevation in ischemic epicardium compared to nonischemic and ischemic endocardium regions. It has been hypothesized that enhanced sympathetic efferent neuronal activity and higher catecholamine levels trigger upregulation of GRK2 and downregulation of cardiomyocyte β-adrenoceptors (Iaccarino et al., 1998). Our data in the ischemic epicardium, but not in the ischemic endocardium, are in accord with this hypothesis. Although this discrepancy between the ischemic endocardium and epicardium has not been characterized before, we hypothesize that the tissue damage in the endocardium generated by ischemia is so profound that it masks downregulation phenomena.

Elevation in GRK2 levels observed in our model may be protective for the cardiac tissue. In transgenic mice overexpressing GRK2 and subjected to 20 min of ischemia followed by 40 min of reperfusion, better recoveries of function, ATP, and phosphocreratine levels are similar to tissue from wild-type mice (Cross et al., 1999). On the other hand, it has been found that a GRK2 peptide inhibitor increase basal contractility without increasing ischemic injury (Cross et al., 1999). It has been also observed that GRK2 peptide inhibitor prevents the development of cardiomyopathy in a mouse model and restores β-adrenoceptor hemodynamic responsiveness (Rockman et al., 1998). Overall, these results suggest that high level of GRK2 may be protective during early reperfusion, but not necessary for longer periods of time.

Forskolin-induced adenylyl cyclase activation was reduced in the ischemic myocardium. This correlated with regional myocardial blood flow measured at 60 min of ischemia. These results suggest that the ischemic insult generated damage at the adenylyl cyclase level which was reflected 72 h after the onset of reperfusion. In the presence of short periods of reperfusion (15–20 min), studies have revealed a reduction of adenylyl cyclase activity induced by NaF or forskolin, suggesting that postreceptor stimulation of adenylyl cyclase is affected (Karliner et al., 1989; Vatner et al., 1993). These results indicated that any reduction of adenylyl cyclase activity in the ischemic region occurs rapidly after the onset of reperfusion and persists for longer period of time.

Using forskolin-induced adenylyl cyclase activity as a covariate for isoproterenol-induced adenylyl cyclase activity indicated that the intensity of the activation of adenylyl cyclase in our experimental model was directly related to the state of the enzyme and not the stimulus. In this experimental protocol, reduction of β-adrenoceptor density appeared to be less important than any attenuation of the adenylyl cyclase to produce cAMP. Whether this is due to a reduction of adenylyl cyclase density or an effect on the active site remains unknown, but our data suggest that only a minor population of the cardiac β-adrenoceptor is needed to maximally activate adenylyl cyclase. Further studies are needed to clarify this issue.

One limitation of this study is that we do not know what types of cells are affected. Cardiomyocytes are affected by ischemia, but other cell types may also be involved. Migrating macrophages or platelets accumulate in the ischemic portion of the myocardium (Rousseau et al., 1993; Yu et al., 2003) and may contribute to modulation of the β-adrenoceptor responsiveness. However, accumulation of these cells occurs mainly in the ischemic myocardium, where the β-adrenoceptor responsiveness is reduced. Another kind of

cell type that may be involved in cardiac β-adrenoceptor responsiveness are the intrinsic efferent postganglionic cardiac neurons. It is known that major cardiac nerves possess their own rich blood supply, much of which arises from extracardiac arteries and are not necessary affected when underlying myocardial tissue becomes ischemic (Janes et al., 1987). Partial preservation of adrenergic nerve function could explain the presence of catecholamine in the ischemic endocardium after 90 min of ischemia. Finally, endothelial cells possess β-adrenoceptors and are more resistant to ischemia than cardiomyocytes (Kloner et al., 1980; Silverman et al., 1995). The surviving endothelial cells in the ischemic regions may influence β-adrenoceptor responsiveness as well.

In summary, after 90 min of ischemia and 72 h of reperfusion, myocardial adenylyl cyclase activity and β-adrenoceptor density correlate with the adequacy of the myocardial blood flow observed during the ischemic period. β-adrenoceptor density in the viable ischemic tissue is modified by the presence of GRK2 and tissue catecholamine, the latter being an index of regional sympathetic efferent post-ganglionic nerve terminal activity.

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