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Metoprolol attenuates postischemic depressed myocardial function in papillary muscles isolated from normal and postinfarction rat hearts

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Abstract

The present study was designed to test the hypothesis that metoprolol treatment may enhance tolerance to ischemia in normal and postinfarction rat myocardium. Myocardial infarction was induced by permanent ligation of the left coronary artery in adult rats. Animals were divided into sham-operated and infarction groups with or without metoprolol treatment. Metoprolol treatment (60 mg/kg/day via gastric gavage) was started on the second day after surgery and continued until sacrifice at 6 weeks after myocardial infarction. Isometric force and intracellular Ca^{2+} ($[Ca^{2+}]_i$) transients were simultaneously recorded in isolated left ventricular papillary muscles. Ischemia was simulated by immersing the muscles into fluorocarbon with hypoxia. Metoprolol treatment induced a significant improvement of isometric force and ameliorated diastolic $[Ca^{2+}]_i$ overload in postinfarction rat myocardium at baseline. Metoprolol treatment also reduced diastolic $[Ca^{2+}]_i$, ameliorated the depression of developed tension during ischemia, and enhanced recovery of postischemic depressed myocardial function in sham-operated and postinfarction rat papillary muscles. Protein levels of the sarcoplasmic reticulum Ca^{2+} ATPase of left ventricles and postischemic papillary muscles from metoprolol-treated rats were higher than those in placebo-treated animals. We concluded, therefore, that metoprolol treatment produced appreciable improvement of intracellular Ca^{2+} handling during ischemia–reoxygenation cycles, and enhanced recovery of postischemic depressed myocardial function in both normal and postinfarction rat myocardium. © 2001 Published by Elsevier Science B.V.

Keywords: Metoprolol; Ca^{2+}, intracellular; Ischemia; Myocardial infarction

1. Introduction

Impaired intracellular Ca^{2+} homeostasis during brief periods of ischemia has been proposed as a primary cause of postischemic myocardial stunning (Kihara et al., 1989; Kusuoka et al., 1987). Ischemia produces high concentrations of catecholamines in myocardial interstitium (Wollenberger and Shahab, 1965) due to exocytotic and non-exocytotic release of norepinephrine from adrenergic nerve endings, and impaired re-uptake mechanisms (Dart and Riemersma, 1985; Karwatowska-Krynska and Beresewicz, 1983; Schomig et al., 1984). This excessive amount of catecholamine release during ischemia is believed to be one of the leading causes of myocardial damage mediated through β-adrenoceptor stimulation. β-adrenoceptors are the regulatory components of the adenylate cyclase-cyclic AMP (cAMP) system. Using isolated myocytes, Mann et al. (1992) showed that adrenergic stimulation led to cAMP-mediated Ca^{2+} overload of the cardiocytes with a resultant decrease in cell viability. These deleterious effects were significantly attenuated by β-adrenoceptor blockade (Mann et al., 1992). Reoxygenation injury may also occur after sudden transmembrane Ca^{2+} influx mediated via increased catecholamine release (Harding and Poole-Wilson, 1980). It has been demonstrated that release of a large quantity of endogenous catecholamine occurs during the early phase of reperfusion (Dart and Riemersma, 1985). Reducing myocardial catecholamine reserves, with either 6-hydroxydopamine or reserpine pretreatment, has been shown to decrease the incidence of reperfusion-induced arrhythmias (Scheridan et al., 1980; Thandroyen et al., 1983). Accordingly, it is tempting to suggest that stimulation of inward Ca^{2+} current by catecholamine exacerbates cellular damage during reoxygenation. In addition, studies in humans suggested that cardiac catecholamine spillover from the heart is significantly increased in patients with cardiac failure at rest (Hasking et

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al., 1986), and that there is further increase during exercise (Hasking et al., 1988). Our previous study reported that postinfarction rat myocardium showed enhanced susceptibility to ischemia–reperfusion injury (Min et al., 2000). Exacerbation of postischemic depressed myocardial function in this model may be caused by excessive release of catecholamines and subsequent impairment of Ca^{2+} homeostasis.

Despite these advances in our understanding of ischemic pathophysiology, relatively little is known about the efficacy and the possible mechanisms of beneficial effects from β-adrenoceptor blockade in normal and postinfarction hearts. The present study was designed to investigate the effects of chronic β-adrenoceptor blockade on postischemic depressed myocardial function in papillary muscles isolated from normal and postinfarction rat hearts. The simulated ischemia model was induced by immersion of the papillary muscle into hypoxic fluorocarbon. This method has made it possible to study myocardial function and intracellular Ca^{2+} handling during ischemia and reperfusion using ferret (Bing et al., 1993) and rat (Min et al., 2000) papillary muscles in our laboratory.

2. Material and methods

2.1. Animal preparation

The experiments were performed in male Wistar rats (Charles River, Wilmington, MA, USA) with an initial body weight of 250–300 g. Animals were housed individually under climate-controlled conditions with a 12-h light/dark cycle and provided with standardized rat chow and tap water ad libitum. This study was conducted under the guidelines for the *Care and Use of Laboratory Animals* published by the US national Institutes of Health (NIH Publication No. 85-23, revised 1996).

Myocardial infarction was induced by ligation of the left anterior descending coronary artery according to a previously described technique (Min et al., 1999). The sham-operated rats underwent an identical surgical procedure without coronary ligation. Animals were randomized to receive either metoprolol (60 mg/kg/day) or placebo by oral gavage once daily starting the second day after surgery. This dosage of metoprolol had previously been shown to produce significant β-adrenoceptor blockade in the rat myocardial infarction model of heart failure (Cherng et al., 1994). The dose of drug was calculated with body weight of each animal monitored weekly. The regimen was continued in the surviving animals until sacrifice at 6 weeks postinfarction.

2.2. Isometric muscle performance

Six weeks after surgery, the animals were anesthetized with pentobarbital. Hemodynamic measurements in vivo were performed with the methods described previously (Litwin and Morgan, 1992). After hemodynamic measurements, the heart was rapidly excised and placed in a dissecting chamber containing a modified Krebs–Henseleit solution of the following composition (mM): NaCl 120, KCl 5.9, dextrose 5.5, $NaHCO_3$ 25, NaH_2PO_4 1.2, $MgCl_2$ 1.2, $CaCl_2$ 1.0, pH 7.4, bubbled with a mixture of 95% O_2 and 5% CO_2 at room temperature. The non-infarcted left ventricular papillary muscle was carefully dissected and then fixed to a muscle holder with a spring clip. The isometric contraction of the papillary muscle was measured with the technique described previously (Min et al., 1999, 2000). The following isometric contraction parameters were recorded from each muscle: developed tension (tension produced by the stimulated muscle), time to peak tension (time from the beginning of the contraction to peak tension) and time to 90% relaxation (time from peak tension to 90% of relaxation). Subsequently, the loading procedure for aequorin was performed (see below). At the end of the experiment, the muscle was blotted and weighed. The cross-sectional area was determined from muscle weight and length by assuming a uniform cross-section and a specific gravity of 1.05. After removal of the papillary muscle for studying, the weights of the right and left ventricles (including the septum) were normalized by body weight and used as indices of hypertrophy. In addition, infarct sizes were quantified according to the method described elsewhere (Pfeffer et al., 1985) in another animal cohort with either placebo or metoprolol treatment (eight for each).

2.3. Aequorin light signal measurement

Aequorin (Dr. John Blinks, Friday Harbor Lab., San Juan Island, WA, USA) was loaded by macroinjection technique (Kihara and Morgan, 1989). The analog signals from the isometric force transducer and electronic photometer were recorded with a chart-strip recorder (Model 56-1X 40-006158, Gould Instrument Systems, Cleveland, OH, USA). Parameters derived from the signals included the amplitude of the light transient, time to peak light and time from peak to 90% decline in light transient. The free intracellular concentration of calcium ($[Ca^{2+}]_i$) was estimated by normalizing the recorded light signal during the isometric twitch by the maximal amount of light emitted after lysis of the muscle membranes at the end of experiment with a 5% solution of the detergent Triton X-100 in phosphate-free physiological salt solution containing 50 mM Ca^{2+}. The normalized light signal was then converted to $[Ca^{2+}]_i$ using an in vitro calibration curve as previously reported (Kihara et al., 1989; Kihara and Morgan, 1989).

2.4. Experimental protocol

Steady-state conditions were observed for at least 30 min. After measuring the baseline parameters in physiological salt solution (PSS) with oxygenation (95% O_2 and

Table 1
General characteristics and hemodynamics of sham-operated and myocardial infarction rats
Values are means ± S.D.

	Sham	Sham + Metoprolol	Infarction	Infarction + Metoprolol
BW (g)	410.8 ± 13.2	406.3 ± 10.6	386.2 ± 9.8	390.3 ± 11.4
LVW (mg)	750.3 ± 20.6	734.8 ± 14.4	865.6 ± 21.7[a]	804.6 ± 13.6
LVW/BW (mg/g)	1.81 ± 0.17	1.79 ± 0.13	2.38 ± 0.20[a]	1.97 ± 0.15
RVW (mg)	273.6 ± 14.1	256.4 ± 13.6	306.8 ± 16.2[a]	280.8 ± 14.6
RVW/BW (mg/g)	0.69 ± 0.12	0.66 ± 0.08	0.84 ± 0.10[a]	0.73 ± 0.09
CSA (mm^2)	0.73 ± 0.08	0.74 ± 0.10	0.96 ± 0.11[a]	0.84 ± 0.09
LVSP (mm Hg)	136.2 ± 6.4	130.8 ± 6.8	83.6 ± 4.7[b]	90.7 ± 5.2[a]
LVEDP (mm Hg)	10.2 ± 0.8	9.8 ± 0.6	21.6 ± 1.7[b]	14.8 ± 1.3[a,c]
$+dP/dt$ ($mg/g \times 10^3$)	8.3 ± 0.6	8.1 ± 0.6	5.5 ± 0.7[b]	6.8 ± 0.9[a,c]
Infarct size (%)			42.6 ± 4.1	40.5 ± 3.8

Sham, sham-operated rat papillary muscles with 6 weeks placebo treatment. Sham + Metoprolol, sham-operated rat papillary muscles with 6 weeks metoprolol treatment. Infarction, postinfarction rat papillary muscles with 6 weeks placebo treatment. Infarction + Metoprolol, postinfarction rat papillary muscles with 6 weeks metoprolol treatment. BW, body weight. LVW, left ventricular weight. RVW, right ventricular weight. LVW/BW, ratio of left ventricular weight/body weight. RVW/BW, ratio of right ventricular weight/body weight. CSA, papillary muscle cross-sectional area. LVSP: the left ventricular systolic pressure. LVEDP: the left ventricular end-diastolic pressure. $+dP/dt$, the rate of peak left ventricular systolic pressure rise. $N = 8$ in each group.

[a] $P < 0.05$.

[b] $P < 0.01$ vs. Sham or Sham + Metoprolol.

[c] $P < 0.05$ vs. Infarction.

5% CO_2), isolated papillary muscles were exposed to oxygenated fluorocarbon (Fluorinert (FC-47), 3M, St. Paul, MN, USA) and equilibrated for a 20-min period. Ischemia was induced by exposing muscle preparations to a 20-min period of hypoxia (95% N_2 and 5% CO_2) with fluorocarbon immersion. Subsequently, reoxygenation was repeated in papillary muscles with fluorocarbon for 30 min. Finally, the bath solution was switched back to physiological salt solution.

2.5. Western blot analysis

The protein levels of the sarcoplasmic reticulum Ca^{2+} ATPase (SERCA2) and phospholamban were measured in additional left ventricles at baseline (five for each group), and rat papillary muscles (five for each group) after ischemia–reoxygenation cycles; i.e., at the state of postischemia from sham-operated and postinfarction rat with or without metoprolol treatment. Briefly, samples were homogenized at 4°C in 4 volumes of 150 mmol/l NaCl. Protein concentrations were determined with a modified Bradford reaction (Bio-Rad, Hercules, CA, USA) using bovine serum album as a standard. Equal amount of total protein (100 μg/lane) were electrophoresed and separated on 10% Pro-cast gel (Novex Electrophoreses, San Diego, CA, USA). Separated proteins were transferred to nitrocellulose membranes blocked in 5% (wt/vol) nonfat dry milk in phosphate buffered saline (PBS). After the mem-

Table 2
Parameters of mechanical contractility and intracellular Ca^{2+} concentration in isolated papillary muscles from sham-operated and myocardial infarction rats
Values are means ± S.D.

	Sham	Sham + Metoprolol	Infarction	Infarction + Metoprolol
Development tension (mN/mm^2)	12.8 ± 1.7	11.9 ± 1.5	9.2 ± 1.3[a]	11.6 ± 1.4[b]
Resting tension (mN/mm^2)	0.7 ± 0.08	0.8 ± 0.09	1.5 ± 0.1[c]	1.0 ± 0.1[b]
Time to peak tension (ms)	98.6 ± 10.4	102.3 ± 10.6	128.6 ± 13.1	117.5 ± 11.4
Time from peak tension to 90% relaxation (ms)	132.6 ± 14.7	121.8 ± 13.2	183.9 ± 18.7[a]	157.6 ± 15.3[b]
Systolic $[Ca^{2+}]_i$ (μM)	0.63 ± 0.07	0.59 ± 0.06	0.62 ± 0.08	0.57 ± 0.03
Diastolic $[Ca^{2+}]_i$ (μM)	0.28 ± 0.02	0.27 ± 0.03	0.34 ± 0.03[a]	0.29 ± 0.02[b]
Time from peak to 90%	48.2 ± 5.8	50.3 ± 5.2	63.5 ± 4.6[a]	54.6 ± 4.9
Decline in light transient (ms)	75.3 ± 6.2	74.6 ± 5.8	90.2 ± 6.3[a]	78.9 ± 5.4[b]

Systolic $[Ca^{2+}]_i$, peak systolic free intracellular Ca^{2+} concentration. Diastolic $[Ca^{2+}]_i$, diastolic free intracellular Ca^{2+} concentration. Sham, sham-operated rat papillary muscles with 6 weeks placebo treatment. Sham + Metoprolol, sham-operated rat papillary muscles with 6 weeks m1toprolol treatment. Infarction, postinfarction rat papillary muscle with 6 weeks placebo treatment. Infarction + Metoprolol, postinfarction rat papillary muscles with 6 weeks metoprolol treatment. $N = 8$ in each group.

[a] $P < 0.05$.

[b] $P < 0.05$ vs. Infarction.

[c] $P < 0.01$ vs. Sham and Sham + Metoprolol.

brane was rinsed, it was separately incubated overnight with a primary single antibody (SERCA2 anti-mouse monoclonal antibody or phospholamban anti-mouse monoclonal antibody, 1:1000 dilution, Affinity Bioregents, Golden, CO, USA). After being rinsed in PBS, the membrane was incubated with peroxidase-labeled mouse antibodies to IgG. Antibody reactions were developed with an enhanced chemiluminescence detection system (ESL, Amersham, USA) and exposed to Kodak MR film for 40–60 s. The relative binding of the antibody to the SERCA2 and phospholamban was determined densitometrically using a NIH imaging system.

2.6. Statistical analysis

Data are expressed as means ± S.D. Results are analyzed by a factorial one-way analysis of variance (ANOVA). Baseline data were analyzed using Student's *t*-test for unpaired data. Intergroup comparisons were made with a Student–Newman–Keuls multiple comparison tests. For ischemia–reoxygenation cycle studies, two-way ANOVA was also used for intergroup comparisons. Significance was defined at the level of $P < 0.05$.

3. Results

Surgical mortality was approximately 10% in the first 24 h. No animals died during the treatment period. The study cohort was comprised of eight animals in each of the following groups: placebo-treated sham-operated rats (Sham); metoprolol-treated sham-operated rats (Sham + Metoprolol); placebo-treated rats with myocardial infarction (Infarction); metoprolol-treated rats with myocardial infarction (Infarction + Metoprolol). Placebo-treated infarction rats had evidence of significant right ventricular hypertrophy, reflected as an increase of right ventricular weight and the ratio of right ventricular weight-to-body

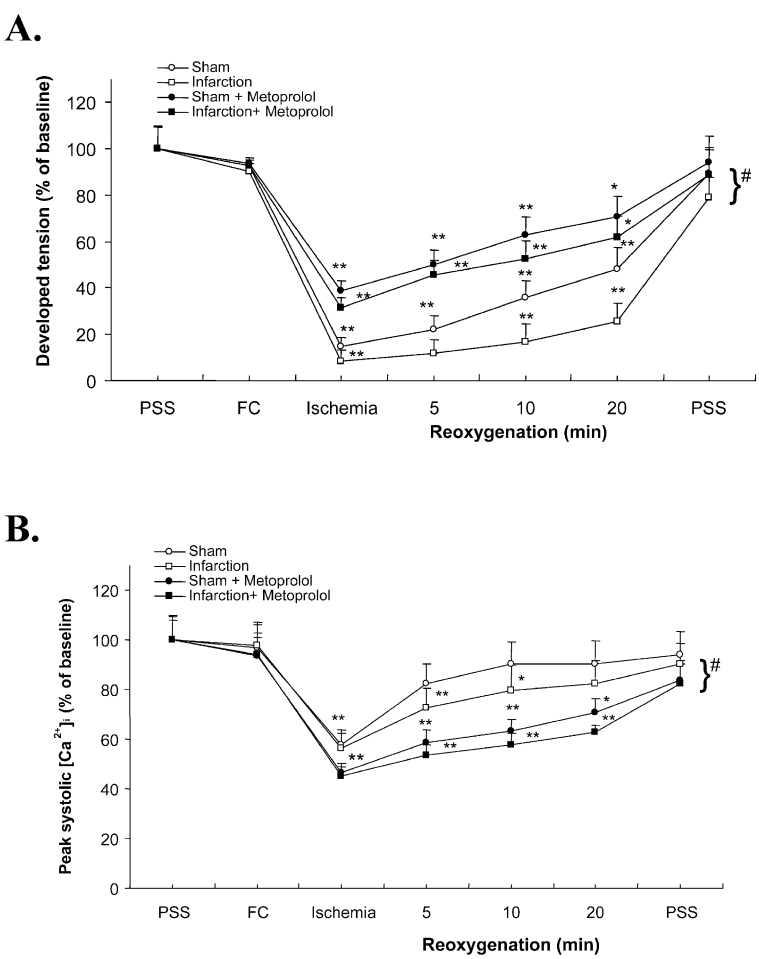

Fig. 1. Developed tension (A) and peak systolic $[Ca^{2+}]_i$ concentration (B) in response to fluorocarbon simulated ischemia–reoxygenation cycle in papillary muscles from sham-operated and myocardial infarction rats with either placebo or metoprolol treatment ($n = 8$ in each group). Sham, sham-operated rat papillary muscles; Sham + Metoprolol, sham-operated rat papillary muscles with metoprolol treatment. Infarction, postinfarction rat papillary muscles with 6 weeks placebo treatment. Infarction + Metoprolol, postinfarction rat papillary muscles with 6 weeks metoprolol treatment. PSS, baseline value in the physiological salt solution with oxygenation. FC, value in the fluorocarbon immersion with oxygenation. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. baseline value in the physiological salt solution. $^{\#}P < 0.05$ Sham vs. Sham + Metoprolol or Infarction vs. Infarction + Metoprolol.

weight (Table 1). Left ventricular weight and left ventricular weight-to-body-weight were elevated in infarction rats compared to the Sham and Sham + Metoprolol groups. Papillary muscle cross-sectional area was increased in postinfarction animals compared to sham-operated rats. However, metoprolol treatment reduced the development of ventricular hypertrophy and also attenuated the papillary muscle cross-sectional area in postinfarction rats as compared to placebo-treated infarction animals. In addition, metoprolol treatment lowered left ventricular end-diastolic pressure, and increased peak rate of left ventricular systolic pressure rise.

3.1. Preischemic muscle mechanics

Papillary muscles isolated from postinfarction rats with placebo treatment had a moderate depression of contractility. The time courses of the contraction and the Ca^{2+} transient were prolonged in muscles from postinfarction rats compared to sham-operated animals (Table 2). Metoprolol treatment produced a slight depression of myocardial contractility in sham-operated rats, but this difference was not significant. Isometric performance of papillary muscles isolated from postinfarction rat hearts was improved with metoprolol therapy. The relaxation of papillary muscle contraction and time to 90% decline of the Ca^{2+} transient were partially normalized in muscle preparations from postinfarction rats treated with metoprolol (Table 2). Under preischemic conditions, the diastolic $[Ca^{2+}]_i$ was significantly elevated in papillary muscles isolated from placebo-treated infarction animals compared to normal muscle preparations from sham-operated rats (Table 2). Metoprolol treatment reduced the increased diastolic $[Ca^{2+}]_i$ in papillary muscles from postinfarction rats.

3.2. Ischemia and postischemic condition

After baseline measurement, papillary muscles were exposed to a 20-min period of immersion in fluorocarbon bubbled with 95% O_2 and 5% CO_2. No significant changes of isometric contractility and intracellular Ca^{2+} were found in four experimental groups with fluorocarbon (Figs. 1–3). Ischemia was produced by a 20-min immersion of papil-

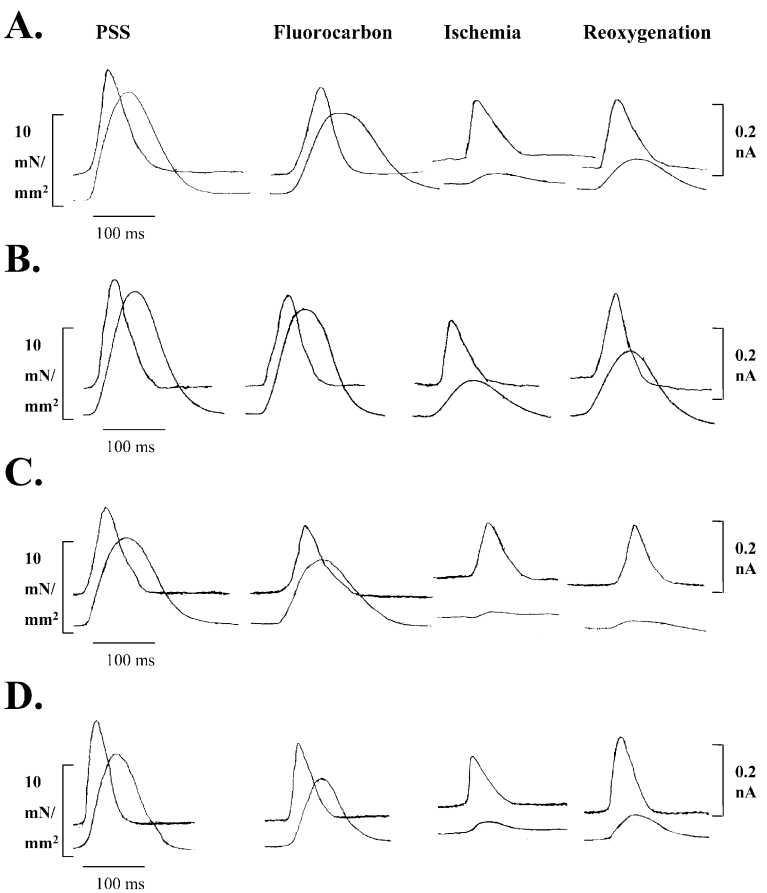

Fig. 2. Aequorin light signal and isometric contraction from representative rat papillary muscles isolated from a sham-operated rat with placebo (A) or metoprolol treatment (B), as well as a postinfarction rat with 6 weeks placebo (C) or metoprolol treatment (D) during fluorocarbon simulated ischemia–reoxygenation cycle. Upper trace of each panel: aequorin light signal. Lower trace of each panel: isometric contraction.

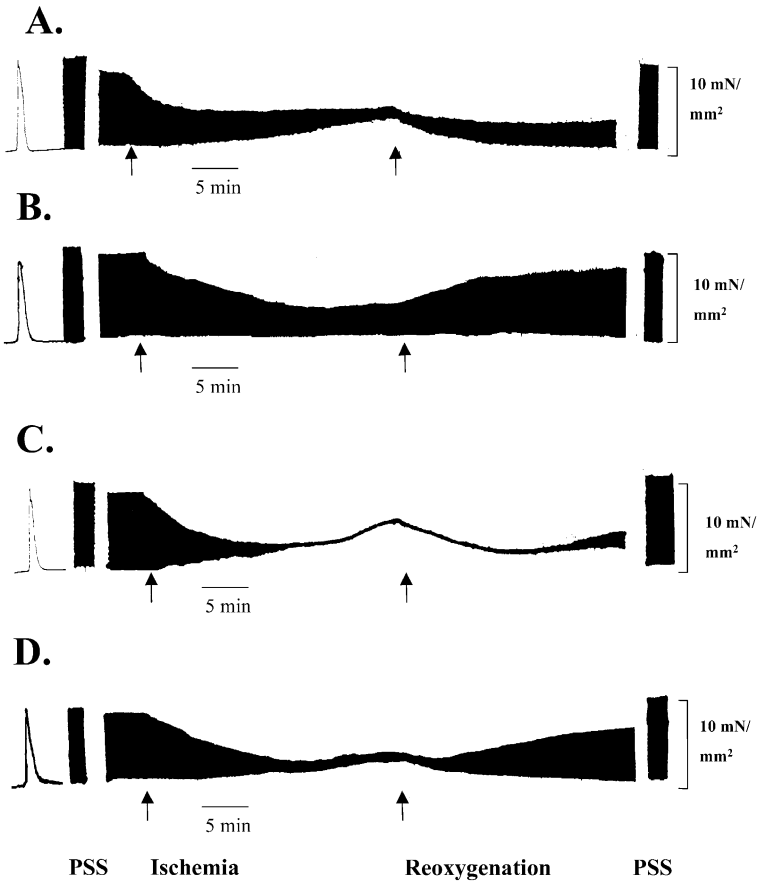

Fig. 3. Continuous chart strip recordings of isometric force in rat papillary muscles isolated from a sham-operated rat with placebo (A) or metoprolol treatment (B), and a postinfarction rat with placebo (C) or metoprolol treatment (D) during fluorocarbon simulated ischemia–reoxygenation cycle. PSS, muscle preparations in the physiological salt solution with oxygenation.

lary muscles in fluorocarbon bubbled with a mixture of 95% N_2 and 5% CO_2. The isometric force was reduced and the resting tension was elevated without a parallel reduction of peak $[Ca^{2+}]_i$ during ischemia (Figs. 2 and 3), and the effect was most pronounced in postinfarction rat myocardium after placebo treatment (Figs. 2C and 3C). Diastolic $[Ca^{2+}]_i$ showed a significant increase after 20 min of ischemia in both sham and the infarction groups with placebo treatment (from 0.28 ± 0.02 to 0.32 ± 0.03 μM in Sham, $P < 0.05$; and from 0.34 ± 0.03 to 0.40 ± 0.04 μM in infarction rat hearts, $P < 0.05$). However, the elevated resting tension and diastolic $[Ca^{2+}]_i$ during ischemia in both sham-operated and postinfarction rat papillary muscles isolated from animals that received metoprolol were significantly attenuated (Resting tension: 2.1 ± 0.4 mN/mm^2 in Sham vs. 0.8 ± 0.09 mN/mm^2 in Sham + Metoprolol, $P < 0.01$; and 4.6 ± 0.8 mN/mm^2 in Infarction group vs. 1.5 ± 0.3 mN/mm^2 in the group of Infarction + Metoprolol, $P < 0.01$). The degree of reduced isometric contraction in muscle preparations isolated from metoprolol-treated sham and postinfarction rats during ischemia was less than that in papillary muscles isolated from sham-operated and postinfarction animals with placebo treatment (Fig. 3).

Postischemic depression of myocardial function was found in all four groups with or without metoprolol treatment. The degree of postischemic depression of myocardial function was significantly greater in the infarction group (Figs. 2C and 3C). Treatment with metoprolol resulted in a similar reversion of postischemic depression of myocardial function in sham-operated and postinfarction rat papillary muscles. Diastolic $[Ca^{2+}]_i$ was significantly decreased in papillary muscles from sham and postinfarction rats with metoprolol treatment compared to placebo-treated animals (0.32 ± 0.03 μM in Sham vs. 0.28 ± 0.02 μM in Sham ± Metoprolol, $P < 0.05$; and 0.40 ± 0.04 μM in Infarction group vs. 0.35 ± 0.03 μmol in the group of MI ± Metoprolol, $P < 0.05$). The increased resting tension during ischemia rapidly recovered to near basal levels after reoxygenation of fluorocarbon in sham-operated and metoprolol-treated postinfarction rat papillary muscles (Fig. 3).

The time courses of isometric contraction and the Ca^{2+} transient were mildly prolonged and peak force reduced in fluorocarbon, but these changes were not significant compared to the values in oxygenated physiological salt solution. We also did not find any significant change in time to peak tension and time from peak tension to 90% relaxation

during further ischemia–reperfusion cycles (data not shown). Whereas time from peak to 90% decline in light transient was significantly prolonged after 20-min ischemia, and it was more pronounced in the infarction group than that in the sham group. With reoxygenation in fluorocarbon, time from peak tension to 90% relaxation recovered promptly to preischemic levels. However, the time from peak to 90% decline in light transient remained prolonged after 30 min of reoxygenation compared to preischemic values in the four groups (Fig. 4B). The prolongation of the time courses of relaxation or 90% decline of Ca^{2+} transient during the postischemic period was less in muscle preparations isolated from sham and postinfarction rats with metoprolol treatment compared to those in papillary muscles isolated from sham and postinfarction rats with placebo treatment (Fig. 4). This phenomenon was much more pronounced in postinfarction rats with metoprolol treatment. When fluorocarbon was replaced by physiological salt solution, the isometric force, $[Ca^{2+}]_i$ availability and time courses of mechanical performance and Ca^{2+} transients promptly recovered to near baseline levels. This observation suggests that fluorocarbon per se did not exert any effect on the characteristics of muscle preparations, and can be used as an appropriate model of simulated ischemia model.

3.3. Protein levels of SERCA2 and phospholamban

The effects of postinfarction remodeling and metoprolol treatment on the levels of SERCA2 and phospholamban expression were measured in additional left ventricles (five each) from sham-operated, placebo-treated and metoprolol-treated postinfarction rats. Expression levels of all genes were analyzed as the ratio compared to cyclophilin. As shown in Fig. 5A, there was a moderate reduction of protein levels of SERCA2 in placebo-treated postinfarction left ventricles compared with that from sham-operated rats.

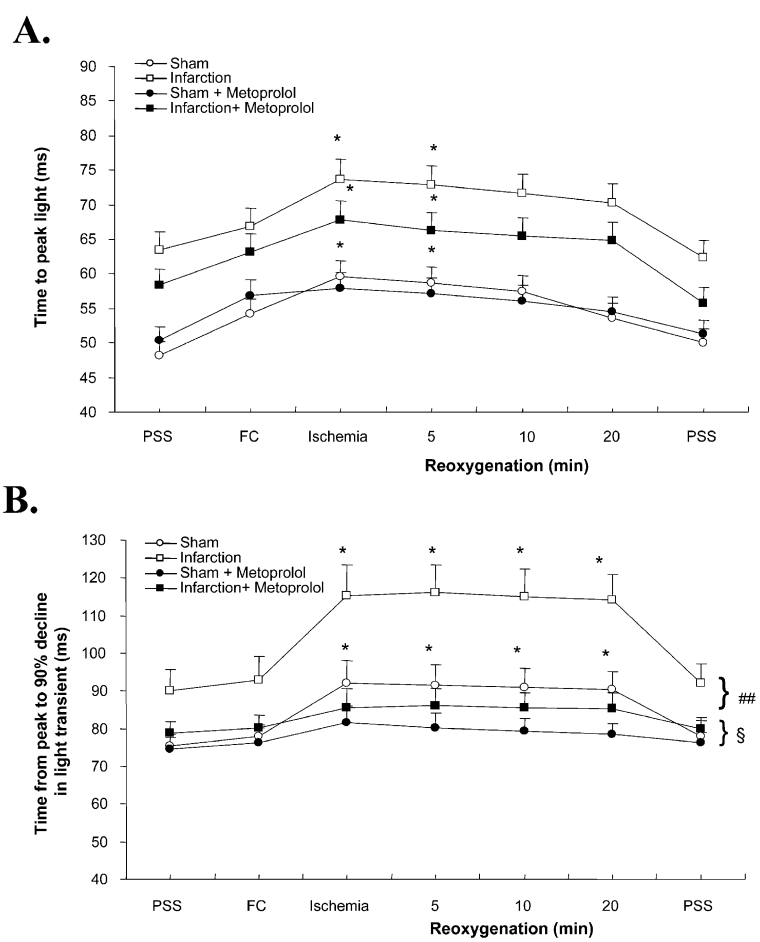

Fig. 4. Time intervals of the intracellular Ca^{2+} transient at baseline and during fluorocarbon simulated ischemia–reoxygenation cycle in the physiological salt solution with papillary muscles isolated from sham-operated and postinfarction rats in either placebo or metoprolol treatment ($n = 8$ in each group). Sham, sham-operated rat papillary muscles with placebo treatment. Sham + Metoprolol, sham-operated rat papillary muscles with placebo treatment. Infarction, postinfarction rat papillary muscles with 6 weeks placebo treatment. Infarction + Metoprolol, postinfarction rat papillary muscles with 6 weeks metoprolol treatment. PSS, baseline value in the physiological salt solution with oxygenation. FC, value in the fluorocarbon immersion with oxygenation. $^{*}P < 0.05$ vs. baseline value in the physiological salt solution. $^{\S}P < 0.05$ Sham vs. Sham + Metoprolol. $^{\#\#}P < 0.01$ Infarction vs. Infarction + Metoprolol.

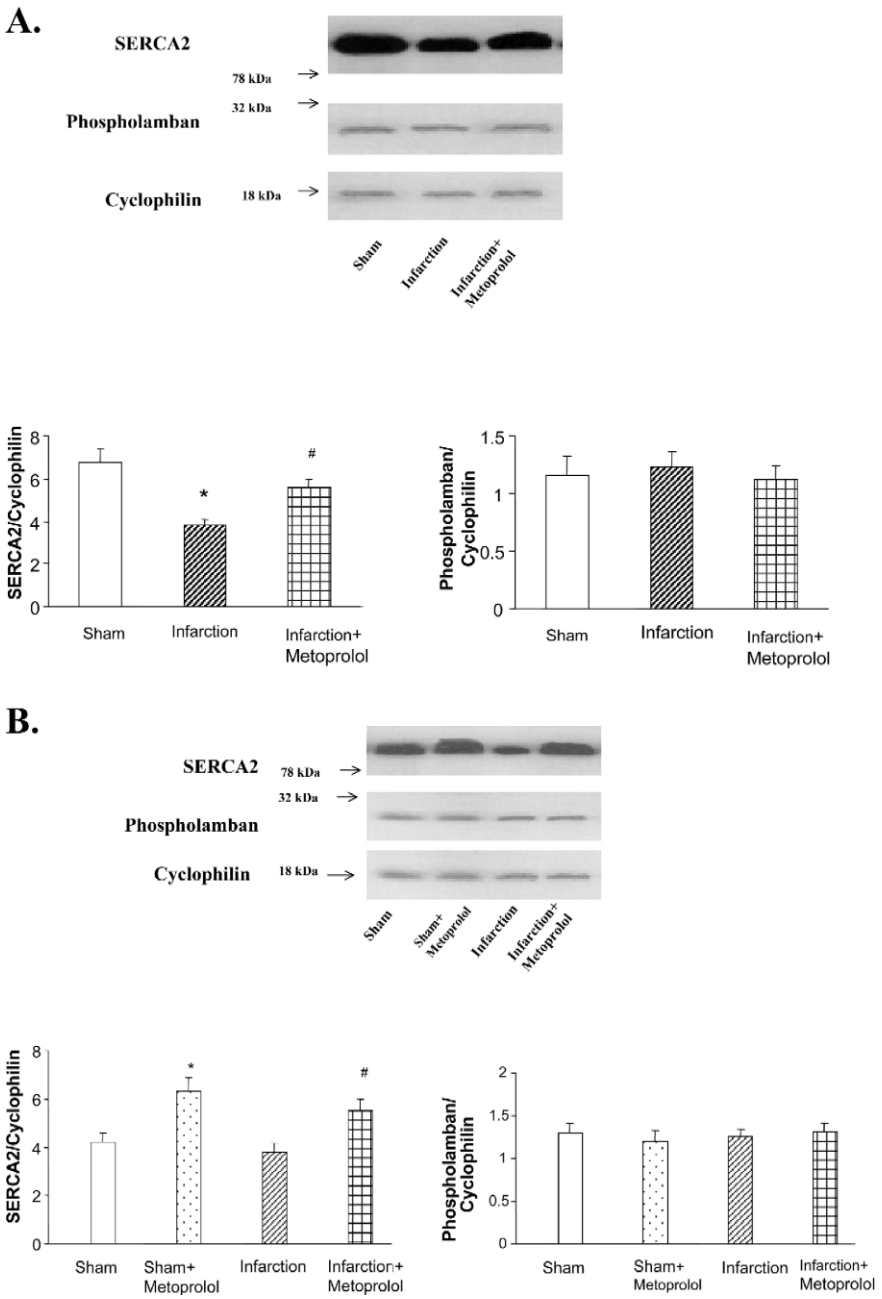

Fig. 5. Protein expressions of SERCA2 and phospholamban in rat left ventricles (A) and isolated papillary muscles at the phase of postischemia (B) were assessed by the Western blot technique. Upper trace of each panel, Western blot. Lower left of each panel, densitometric analysis of SERCA2/Cyclophilin. Lower right of each panel, densitometric analysis of phospholamban/cyclophilin. $N = 5$ in each group. $^{*}P < 0.05$ vs. Sham. $^{\#}P < 0.05$ vs. Infarction.

In contrast, SERCA2 protein levels underwent less of a decrease in left ventricles from postinfarction rats after metoprolol treatment vs. placebo-treated postinfarction animals. After the simulated ischemia–reoxygenation cycle with fluorocarbon immersion, SERCA2 and phospholamban were measured in four additional groups of postischemic papillary muscles from Sham, Sham + Metoprolol, Infarction and Infarction + Metoprolol rat hearts in order to demonstrate the levels of Ca^{2+} regulatory protein levels during the phase of postischemia. Samples were obtained after 30 min of reoxygenation in fluorocarbon. SERCA2 protein levels were decreased in papillary muscles at the phase of postischemia from sham-operated and postinfarction rats with placebo treatment compared to those from sham-operated and postinfarction rat papillary muscles with metoprolol treatment (Fig. 5B). In contrast to SERCA2, the protein levels of phospholamban were similar in left ventricles as well as postischemic papillary muscles from Sham, Sham + Metoprolol, Infarction and Infarction + Metoprolol groups.

4. Discussion

The main findings of the present study are: (1) metoprolol treatment partially prevents development of abnormal intracellular Ca^{2+} handling with significant improvement of isometric contraction in postinfarction rat myocardium at baseline; (2) more severe alteration of intracellular Ca^{2+} modulation in postinfarction rat myocardium decreases tolerance to fluorocarbon-simulated ischemia, with consequently more severe postischemic depressed myocardial function; and (3) metoprolol treatment attenuates increased resting tension and elevated diastolic intracellular Ca^{2+} during ischemia, and subsequently results in partial recovery of postischemic depressed myocardial function in normal as well as postinfarction rat myocardium.

4.1. Effect of metoprolol on baseline conditions

Abnormal intracellular Ca^{2+} handling has been suggested as a major cause of contractile dysfunction in failing myocardium (Morgan et al., 1990; Morgan, 1991). The descending phase of the Ca^{2+} transient predominantly reflects the resequestration of Ca^{2+} by the sarcoplasmic reticulum (Morgan et al., 1984, 1990). Thus, the prolongation of the Ca^{2+} transient in the present study suggests that there are abnormalities of reuptake of Ca^{2+} by the sarcoplasmic reticulum in postinfarction rat myocardium. Increased diastolic Ca^{2+} levels in papillary muscles isolated from postinfarction rats provided further evidence of abnormal handling of intracellular Ca^{2+} in postinfarction myocardium. Long-term metoprolol treatment produced significant shortening in the time to 90% relaxation of isometric contraction and the time from the peak of the Ca^{2+} transient to 90% decline. Furthermore, treatment with metoprolol significantly reduced diastolic $[Ca^{2+}]_i$ in remodeled myocardium from postinfarction rats compared to placebo-treated animals. Therefore, the significant enhancement of myocardial contraction in postinfarction rats is associated with improvement of intracellular Ca^{2+} handling by metoprolol treatment.

There is accumulating evidence to suggest that reduced expression and/or function of SERCA2, as well as increased expression and/or function of phospholamban (Kadambi et al., 1996) are major changes contributing to altered Ca^{2+} homeostasis in the failing hearts from postinfarction rats (Afzal and Dhalla., 1992) and human failing myocardium (Schmidt et al., 1998), and subsequently decrease the reuptake of Ca^{2+} by the sarcoplasmic reticulum. In the present study, left ventricular protein levels of SERCA2 were significantly decreased in placebo-treated postinfarction rats compared to those in sham-operated and metoprolol-treated postinfarction animals, although the protein levels of phospholamban were similar in all groups. Therefore, it is reasonable to speculate that this beneficial effect of ameliorating abnormal handling of intracellular Ca^{2+} with metoprolol treatment is, at least partially, due to enhanced expression of the Ca^{2+} regulated protein SERCA2.

4.2. Effect of metoprolol on postischemic depressed myocardial function

It has been demonstrated that ischemia causes release of large quantities of catecholamines from the myocardium (Dart et al., 1984; Karwatowska-Krynska and Beresewicz, 1983; Wollenberger and Shahab, 1965) through β-adrenoceptor mediated stimulation of cAMP. During the early phase of reperfusion, Dart et al. (1984) found that there is still a large release of endogenous catecholamines. The fact that high concentrations of catecholamines can produce myocardial necrosis has been known for several decades (Waldenstrom et al., 1978). Increased adrenergic activity in the ischemic heart may produce adverse biologic effects on cardiac myocytes (Bristow et al., 1982). The concept that sustained β-adrenergic stimulation of the ischemic heart leads to progressive left ventricular dysfunction is supported by direct evidence that norepinephrine can decrease contractile function and alter gene expression in isolated cardiac myocytes (Mann et al., 1992). In the fluorocarbon-simulated ischemia model, we found that ischemia depressed isometric contraction with similar Ca^{2+} transients and increased resting tension. Those phenomena are more pronounced in postinfarction rat myocardium. Thus, the attenuation of depressed isometric contractility during ischemia and a partial reversion of postischemic depressed myocardial function by metoprolol treatment could be related to blockade of the β-adrenoceptor during the ischemia–reperfusion cycle. However, another study of ischemia showed an increase of intracellular Ca^{2+} (Kusuoka et al., 1987) in contrast to a non-significant change of Ca^{2+} transient in the present study. One of the possible explanations for these differences was glycogen depletion because the response of the Ca^{2+} transient to ischemia is partially dependent on lactate production from glycogen stores; reduced lactate production due to glycogen depletion could explain the failure to observe an increase in intracellular Ca^{2+}. Tissue CO_2 retention may also play a role in increasing intracellular Ca^{2+} during ischemia. In contrast to true ischemia where the pressure of CO_2 may rise to very high level, in fluorocarbon CO_2 is highly diffusible and tissue CO_2 will only increase to the concentration where it will equilibrate with the 5% CO_2 bubbled through the solution (Bing et al., 1993).

In addition to blockade of the β-adrenoceptor during ischemia–reperfusion by metoprolol, the beneficial effects of metoprolol in postischemic myocardial dysfunction may also be related to improved intracellular Ca^{2+} handling. Studies reported by our laboratory (Kihara et al., 1989; Min et al., 2000) and others (Kusuoka et al., 1987) have shown that impaired intracellular Ca^{2+} modulation is a primary cause of myocardial stunning. With the myocardial infarction model, we found a greater alteration of

intracellular Ca^{2+} modulation with impaired $[Ca^{2+}]_i$ homeostasis in postinfarction rat myocardium leads to a decreased tolerance to ischemia–reperfusion injury (Min et al., 2000). Intracellular Ca^{2+} overload in cardiac muscle has been defined as an abnormal rise in intracellular-free Ca^{2+} concentration during diastole (Clusin et al., 1983; Kihara and Morgan, 1991). Dysregulation of diastolic $[Ca^{2+}]_i$ homeostasis has been implicated in the pathophysiology of heart failure (Gwathmey et al., 1987) and cardiac dysfunction of the period of ischemia–reperfusion (Meissner and Morgan, 1995). Thus, increased diastolic tonus and reduced systolic performance were proposed to be manifestations of impaired diastolic $[Ca^{2+}]_i$ regulation (Lakatta, 1989). The present study shows that metoprolol treatment can ameliorate increased resting tension and elevated diastolic $[Ca^{2+}]_i$ during ischemia, subsequently result in partial recovery of postischemic mechanical dysfunction by reduction of intracellular Ca^{2+} overload in both normal and postinfarction rat myocardium.

The mechanisms responsible for the beneficial effect of metoprolol treatment on abnormal intracellular Ca^{2+} handling are unclear. The protein levels of Ca^{2+}-regulated proteins have not yet been reported in the study of postischemic myocardial dysfunction. Excessive amounts of catecholamine during ischemia and further reperfusion may compromise the Ca^{2+} regulatory proteins. Our present finding demonstrated that the ameliorative effects of metoprolol on abnormal intracellular Ca^{2+} handling during ischemia–reperfusion cycle, and subsequent postischemic depression of myocardial function are, at least partially, related to increased gene expression of SERCA2.

In summary, the evidence discussed herein indicates that metoprolol treatment not only has beneficial effects on basal condition contractility and intracellular Ca^{2+} handling in postinfarction rat myocardium, but also can ameliorate postischemic depressed myocardial function by interfering with the mechanism of myocyte injury.

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