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Reversal of Cardiac Remodeling and Subcellular Defects by Prazosin in Heart Failure Due to Myocardial Infarction

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Abstract

Background: This study was undertaken to test if α-Adrenoceptor (AR) blockade with prazosin reverses cardiac remodeling and ameliorates subcellular defects in heart failure due to Myocardial Infarction (MI).

Methods: Heart failure in rats was induced by MI for 12 wks and then treated with or without prazosin (10 mg/kg/day) for 8 wks. Both control and experimental animals were assessed hemodynamically and echocardiographically for evaluating changes in heart function and cardiac remodeling, respectively. Left Ventricle (LV) was used for determining biomedical and molecular activities.

Results: Cardiac dysfunction, as evident from depressed LV systolic pressure, rates of changes in pressure development and decay, cardiac output, ejection fraction and fractional shortening as well as increased LV end diastolic pressure, in 20 wks infarcted animals, was corrected partially by prazosin treatment. Different parameters of cardiac remodeling including increased LV posterior wall thickness and LV systolic diameter in failing hearts were fully or partially reversed whereas increased LV diastolic diameter was unaltered by prazosin therapy. Reversal of lung congestion and cardiac hypertrophy in MI animals by prazosin was associated with depression in the elevated levels of plasma norepinephrine, unlike epinephrine or dopamine. Depressions in Sarcoplasmic Reticular (SR) Ca^{2+}-uptake activity as well as protein content for Ca^{2+}-pump ATPase and phospholamban in failing hearts were reversed partially whereas depressed SR Ca^{2+}-release activity and myofibrillar Ca^{2+}-stimulated ATPase activity were not affected by prazosin. Alterations in mRNA levels for SR Ca^{2+}-pump ATPase, SR Ca^{2+}-release channels, and α-myosin heavy chain and β-myosin heavy chain in MI-induced heart failure were not influenced by prazosin treatment.

Conclusions: It is suggested that the activation of α-AR system may be associated with cardiac remodeling and heart failure and the reverse cardiac remodeling by α-AR blockade may improve cardiac performance by attenuating defects in SR Ca^{2+}-pump.

Keywords: α-Adrenoceptor blockade; Myosin gene expression; Ca^{2+}-pump gene expression; Myofibrillar atpase activity; Ca^{2+}-transport activity

Introduction

Since the positive inotropic effect of α-Adrenoceptor (AR) activation is preserved in failing hearts due to myocardial infarction (MI) [1,2], it is considered that α-ARs play an important role in maintaining cardiac function during the development of heart failure. Neither the α_1-AR density nor the positive inotropic action due to α-AR stimulation was altered in failing human hearts [3,4]. Furthermore, both chronic infusion of norepinephrine (NE) and the elevated levels of plasma NE in heart failure [5], did not reduce the α-AR density in the rat myocardium appreciably [6]. In fact, some investigators have suggested that the activation of α-AR system may serve as an adaptive mechanism in the failing heart [7] and some selective α-AR agonists may represent a novel approach for the treatment of heart failure [8,9]. In this regard, it was observed that the α-AR density was increased and the positive inotropic effect of α-AR activation was depressed in the end-stage failing human heart [10]. On the other hand, α-AR associated mechanisms have been indicated to be intimately involved during the development of heart failure [11]. This view is supported by several clinical studies, which have shown beneficial effects of different α-AR blockers including prazosin in heart failure [12-15]. The combination therapy with prazosin and enalapril (an angiotensin converting enzyme inhibitor) produced greater effects than the monotherapy with enalapril in chronic heart failure [16]. Although the combination therapy of heart failure with prazosin and metoprolol (a β-AR blocker) did not produce any additive effects [17], the outcome in heart failure due to the blockade of both α-AR and β-AR with carvedilol was better than that due to the blockade of β-AR with metoprolol [18]. Blockade of α-ARs in heart failure in dogs was also found to improve coronary flow, myocardial function and metabolism [19]. However, some clinical trials have failed to observe any positive effects of α-AR blockers in heart failure [20,21] and such negative results are considered to be due to differences in the etiology of heart failure, doses of drugs and duration of drug treatment.

Cardiac dysfunction in heart failure is invariably explained on the basis of changes in shape and size of the heart (cardiac remodeling) [22] as well as defects in subcellular organelles such as Sarcoplasmic Reticulum (SR) and Myofibrils (MF) [23-25]. Since the sympathetic nervous system is markedly activated in heart failure [26,27], the elevated levels of plasma catecholamines are known to play a critical role in the pathogenesis of cardiac dysfunction, cardiac remodeling and subcellular alterations [22-25]. Such adverse effects of high levels of circulating catecholamines in heart failure can be seen to be

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mediated through the participation of both α-AR and β-AR in the myocardium. Although β-AR blockers have been demonstrated to attenuate myocardial changes in heart failure [27,28], no information regarding the beneficial effects of α-AR blockade on cardiac remodeling or subcellular defects in failing hearts is available in the literature. This study was therefore undertaken to investigate if the depressed cardiac function in rats with heart failure due to MI is improved and cardiac remodeling is attenuated upon treatment with prazosin, a well-known α-AR blocker. Since both SR and MF are intimately involved in determining the status of cardiac contraction and relaxation [23-25], the activities of SR Ca^{2+}-uptake, SR Ca^{2+}-release and MF Ca^{2+}-stimulated ATPase were examined in MI animals with or without prazosin treatment. Furthermore, to test if the beneficial effects of α-AR blockade on subcellular activities occur at the level of cardiac gene expression [23-25], changes in mRNA levels for both SR and MF proteins were measured in untreated and prazosin treated hearts. Because of the involvement of sympathetic nervous system activation and subsequent elevated levels of plasma catecholamines in heart failure [26,27], the status of plasma NE, Epinephrine (EPI) and dopamine in MI-induced heart failure was monitored upon treatment with prazosin.

Methods

Experimental model

All protocols used in this study were approved by the University of Manitoba Animal Care Committee according to the guidelines established by the Canadian Council on Animal Care. Male Sprague-Dawley rats (6 to 8 wks old) weighing 175-200 g were used in this study. Heart failure due to MI in these animals was induced by occluding the coronary artery according to the procedure described earlier [29,30]. Sham operated animals without any coronary artery occlusion were used as control. Twelve weeks after inducing MI, these experimental animals showed marked depressions in Cardiac Output (CO), Ejection Fraction (EF) and Fractional Shortening (FS) upon echocardiographic examination [31]. These 12 wks MI animals were treated with or without prazosin (10 mg/kg/day; orally) for 8 wks. Sham control rats were also treated with or without prazosin for a period of 8 wks. Prazosin was dissolved in tap water (10 mg/ml) every day and this solution was administered orally by gauge in a volume of 1 ml/kg. At the end of 20 wks, the animals (26 to 28 wks old) were used for morphometric, hemodynamic, cardiac remodeling, and biochemical measurements.

Hemodynamic assessment and measurements of plasma catecholamines

Rats were anaesthetized with an injection of ketamine and xylazine mixture (90:10 mg/kg) and assessed hemodynamically by employing a microtipped pressure transduced catheter (Model SPR-249, Miller Instruments, Houston, TX) [30,32]. Different hemodynamic parameters, such as Mean Arterial Pressure (MAP), Heart Rate (HR), Left Ventricular (LV) Systolic Pressure (LVSP), LV End-Diastolic Pressure (LVEDP), rate of pressure development (+dP/dt), and rate of pressure decay (-dP/dt), were recorded by a computer program (Acqknowledge 3.03, MP 100, BIOPAC Systems, Goleta, CA). At the end of the experiment, blood from LV was withdrawn for preparing plasma. The heart, lung, as well as liver were dissected out, washed and weighed. The scar, Right Ventricle (RV) and Viable LV (Including Septum) were separated and weighed. The scar wt/LV wt ratio was used as an index of scar size whereas heart wt/body wt ratio was taken as an index of cardiac hypertrophy [31,33]. Lung wet wt/dry wt ratio was used as an index for lung congestion whereas liver wet wt/dry wt ratio was used as an index for liver congestion. Plasma catecholamines, NE, EPI, and dopamine were measured [27] using a Bio-Rad plasma catecholamine reagent kit for high performance liquid chromatography (Waters 2690 Separation Module) with electrochemical detection (Amperometric LC-4C detector, Bioanalytical Systems).

Echocardiographic assessment and LV performance

In order to gain information regarding cardiac remodeling and cardiac performance, animals were examined echocardiographically [27,31] using an ultrasound imaging system (Sonos 5500) equipped with 512 phased-array transducer (Agilent Technologies Inc., Andover, MA). The following parameters of cardiac remodeling were recorded: LV Systolic and Diastolic Diameters (LVIDs and LVIDd); intraventricular septum systolic and diastolic thickness (IVSs and IVSd); LV posterior wall thickness in systolic and diastolic modes (LVPWs and LVPWd). The parameters recorded for cardiac performance were: CO, FS, EF, and HR.

SR Ca^{2+}-transport and MF ATPase activities

The viable LV, including septum, was used for the isolation of SR and MF preparations according to the methods used earlier [30,33-35]. The SR Ca^{2+}-uptake and SR Ca^{2+}-release activities, as well as MF Ca^{2+}-stimulated ATPase and MF Mg^{2+}-ATPase activities were determined in both control and experimental preparations [30,33-35]. To show if changes in SR Ca^{2+}-transport activities were associated with alterations in proteins in the SR membranes, protein content for Ca^{2+}-pump (SERCA2a), Phospholamban (PLB), and Calsequestrin (CQS) were determined by Western blot analysis [30,34,35]. For this purpose, SR proteins in different samples (20 μg protein) were separated by SDS-polyacrylamide gel electrophoresis, probed with appropriate antibodies [34] and different bands were detected by using Amersham ECL kit (GE Healthcare UK Ltd., Little Chalfont, Buckinghamshire, UK). Gels were stained with coomassie blue after blotting, and blots were stained with Ponceau S solution to ensure uniform protein loading in all groups. The bands were analyzed by GS-670 Imaging Densitometer (Bio-Rad Laboratories, Mississauga, Ontario) with the Image Analysis Software (version 1.0). The values were expressed as a percentage of sham control.

Northern blot analysis and cardiac gene expression

In order to examine if alterations in SR and MF activities in failing hearts with or without drug treatment occur at the level of gene expression for SR and MF proteins, mRNA levels were determined by isolating total RNA and using Northern blot analysis [30,33-36]. By employing specific molecular probes, mRNA levels for SR Ca^{2+}-pump, SR Ca^{2+}-release channels (RyR), SR PLB, α-myosin heavy chain (α-MHC), and β-myosin heavy chain (β-MHC) proteins were measured; 18S rRNA were used as internal standard.

Statistical analysis

All values were presented as mean ± S.E. Statistical analysis of the data was performed using MicroCal Origin Version 6 and differences between groups were evaluated by one-way analysis of variance (ANOVA) followed by Newman-Keul's test; a P value of <0.05 was considered significant.

Results

General characteristics

It can be seen from Table 1 that MI for a period of 20 wks produced a large scar size and induced marked increases in heart wt, heart wt/

body wt ratio, RV wt and lung wet/dry wt ratio without any changes in scar wt. Treatment of 12 wks MI animals with prazosin for 8 wks revealed a reduction in heart wt from an increase of 56%, to 34% when compared to control hearts. The heart wt/body wt ratio was also partially decreased with prazosin from 54% increase to 27%. A marked (63%) increase in RV wt due to MI was depressed by prazosin to an 8% increase whereas the increase in lung wet wt/dry wt ratio decreased from 30% to 1% below the value of control group. However, prazosin therapy did not affect the scar wt/LV wt ratio or the liver wet wt/dry wt ratio in MI animals. Treatment of control animals with prazosin had no effect on any parameter for general characteristics (Table 1).

Cardiac dysfunction and plasma catecholamines

The infarcted animals exhibited depression in cardiac function, which was evident from a marked increase in LVEDP (3.8 fold) as well as 48% decrease in +dP/d*t*, 58% decrease in –dP/d*t*, and 47% decrease in LVSP, without any significant change in MAP (Table 2A). An improvement in cardiac function in failing hearts due to MI was observed with prazosin treatment; this was revealed by modifications in the following parameters: LVEDP elevation was lowered from 3.8 fold to 1.7 fold, LVSP increased from 53% to 73%, +dP/d*t* increased from 52% to 67%, and -dP/d*t* increased from 42% to 67%. Treatment

Parameter	Sham	MI	Sham + PRAZ	MI + PRAZ
Body wt (g)	685 ± 38	679 ± 27	696 ± 26	653 ± 46
Heart wt (mg)	1720 ± 55	2690 ± 79*	1900 ± 14	2300 ± 7#
Scar wt (mg)	ND	763 ± 125	ND	670 ± 99
Scar wt/LV wt (%)	ND	41 ± 4	ND	39 ± 4
Heart wt/body wt (mg/g)	2.71 ± 0.08	4.20 ± 0.13*	2.73 ± 0.20	3.45 ± 0.18#
RV wt (mg)	386 ± 25	633 ± 75*	267 ± 21	417 ± 31#
Lung wet/dry wt ratio	4.01 ± 0.06	5.23 ± 0.26*	4.26 ± 0.24	3.98 ± 0.25#
Liver wet/dry wt ratio	2.65 ± 0.11	2.91 ± 0.05	2.82 ± 0.03	2.93 ± 0.17

Values are a mean SE of 6 animals in each group. MI – Myocardial Infarction; PRAZ– Prazosin (10 mg/kg/day); ND – Not Detected; LV– Left Ventricle; RV – Right Ventricle;*P < 0.05 compared with the 20 week sham group; #P < 0.05 compared with the 20 week MI group

Table 1: General characteristics of sham and infarcted rats with and without prazosin treatment for 8 weeks starting at 12 weeks after coronary artery occlusion.

Parameter	Sham	MI	Sham + PRAZ	MI + PRAZ
A. Hemodynamic parameters				
Heart rate (bpm)	225 ± 5	234 ± 5	241 ± 6	238 ± 9
LVSP (mm Hg)	139 ± 4	74 ± 3*	138 ± 3	102 ± 3#
LVEDP (mm Hg)	5.1 ± 0.42	19.3 ± 0.61*	5.2 ± 0.30	8.8 ± 0.33#
+dP/dt (mm Hg/s)	7377 ± 390	3840 ± 139*	6924 ± 331	4955 ± 166#
-dP/dt (mm Hg/s)	5600 ± 150	2366 ± 235*	5263 ± 104	3789 ± 311#
MAP (mm Hg)	147 ± 15	131 ± 11	156 ± 13	142 ± 17
B. Plasma catecholamines				
Norepinephrine (pg/ml)	182 ± 6.2	352 ± 11.5*	163 ± 14.7	187 ± 17.3#
Epinephrine (pg/ml)	80 ± 5.0	137 ± 6.3*	80 ± 4.9	141 ± 13.1
Dopamine (pg/ml)	70 ± 8.2	202 ± 15.7*	79 ± 6.2	174 ± 8.8

Values are mean ± SE of 6 animals in each group. LVSP–Left Ventricular Systolic Pressure; LVEDP–Left Ventricular End Diastolic Pressure; MI–Myocardial Infarction; MAP–Mean Arterial Pressure; +dP/dt–rate of pressure development; -dP/dt rate of pressure decay; bpm–beats per min; PRAZ–Prazosin (10 mg/kg/day); *P <0.05 compared with the 20 week sham group; #P <0.05 compared with the 20 week MI group

Table 2: Hemodynamic parameters and plasma catecholamine levels in sham and myocardial infarcted rats with and without prazosin treatment for 8 weeks beginning at 12 weeks post coronary artery occlusion.

Parameter	Sham	MI	Sham + PRAZ	MI + PRAZ
A. Parameters for interval dimensions				
IVSd (cm)	0.251 ± 0.03	0.219 ± 0.02	0.242 ± 0.01	0.271 ± 0.02
IVSs (cm)	0.394 ± 0.03	0.277 ± 0.03*	0.355 ± 0.01	0.418 ± 0.02#
LVIDd (cm)	0.861 ± 0.01	1.100 ± 0.04*	0.875 ± 0.02	1.020 ± 0.06
LVIDs (cm)	0.477 ± 0.03	0.842 ± 0.03*	0.537 ± 0.04	0.643 ± 0.04#
LVPWs (cm)	0.257 ± 0.03	0.372 ± 0.02*	0.246 ± 0.01	0.247 ± 0.01#
LVPWd (cm)	0.350 ± 0.01	0.448 ± 0.03*	0.309 ± 0.02	0.346 ± 0.02#
B. Cardiac performance parameters:				
Ejection fraction (%)	86 ± 3.0	44 ± 2.0*	67 ± 4.0	64 ± 4.0#
Fractional shortening	44 ± 3.9	21 ± 1.5*	33 ± 3.5	32 ± 3.2#
(%)Cardiac output (L/min)	0.479 ± 0.03	0.234 ± 0.03*	0.433 ± 0.05	0.372 ± 0.03#
Heart rate (bpm)	337 ± 8	369 ± 4	336 ± 15	348 ± 7

Values are mean ± SE of 7 animals in each group. MI–Myocardial Infarction; PRAZ–Prazosin (10 Mg/Kg/Day); IVS–Internal Ventricular Septum; LVID–Left Ventricular Internal Diameter; LVPW–Left Ventricular Posterior Wall; d–diastolic measurement; s–systolic measurement. *P<0.05 compared with the 20 week sham group. #P<0.05 compared with the 20 week MI group.

Table 3: Parameters for internal cardiac diastolic and systolic dimensions as well as cardiac performance as measured by echocardiography of sham and infarcted animals with and without prazosin treatment for 8 weeks.

of control animals with prazosin showed no action on cardiac function (Table 2A).

Plasma NE and E levels were considerably elevated in the infarcted rats as compared to the sham rats (1.9 fold increase and 1.7 fold increase, respectively, Table 2B). Treatment of MI rats with prazosin revealed a significant decline in the circulating levels of NE (0.53 fold decrease) without any significant change in E levels. Plasma dopamine levels were also elevated (3.0 fold increase) in the infarcted animals but treatment with prazosin did not reveal any significant reduction in the dopamine levels of the infarcted rats (Table 2B). Treatment of control animals with prazosin had no effect on plasma catecholamines.

Cardiac remodeling and cardiac performance

Table 3A shows cardiac diastolic and systolic echocardiographic parameters of the MI rats to illustrate a 30% decrease in IVSs, 27% increase in LVIDd, 77% increase in LVIDs, 45% increase in LVPWs, and 28% increase in LVPWd. Prazosin treatment showed a reversal in these cardiac modifications, as IVSs increased from 70% to 94%, LVIDs was reduced from 177% to 135%, LVPWs declined from 145% to 96%, and LVPWd decreased from 128% to 99%. However, there was no significant reduction in the LVIDd value upon treatment of infarcted rats with prazosin. There were no significant alterations observed in IVSd in any group. Treatment of control animals with prazosin showed no effect on any parameter for cardiac remodeling (Table 3A). Echocardiographic studies with 20 wks post MI rats also showed reductions in parameters for cardiac performance with 49% decrease in EF, 53% decrease in FS%, and 52% decline in CO (Table 3B). Treatment of MI animals with prazosin revealed partial attenuation of changes in cardiac performance, with increases in EF from 51% to 75%, FS from 47% to 72%, and CO from 48% to 78%. There was no change in HR in MI animals with or without prazosin treatment. Likewise, treatment of control animals with prazosin showed no effect on any parameter for cardiac performance (Table 3B).

Subcellular activities and SR protein content

Table 4 shows decline in SR Ca^{2+}-uptake and Ca^{2+}-release in 20 wks MI rat hearts with 70% and 56% reductions in the activities, respectively. MF from the viable LV of MI animals also showed a decrease in Ca^{2+}-stimulated ATPase activity of 37% when compared to sham control (Table 4). Treatment of MI animals with prazosin improved SR Ca^{2+}-uptake activity partially but significantly. However, therapy of infarcted animals with prazosin did not reveal any effect on altered SR Ca^{2+}-release and MF Ca^{2+}-stimulated ATPase activities. There were no alterations observed in MF Mg^{2+}-ATPase activity in the infarcted hearts with or without prazosin treatment. Similarly, control animals receiving prazosin showed no effect on SR or MF activities (Table 4).

For understanding the mechanisms in changes in SR Ca^{2+}-uptake activity in the failing hearts, protein content of SR, PLB, SERCA2a, and CQS were measured by Western blot analysis (Figure 1A). The MI hearts showed a decline in the expression of PLB by 43% (Figure 1B), SERCA2a by 69% (Figure 1C), without any significant modifications in the protein expression of CQS (Figure 1D). After therapy with prazosin, the infarcted hearts did exhibit an appreciable recovery in the protein expression with an increase from 57% to 76% for PLB and 31% to 81% for SERCA2a, but no apparent change in the values for CQS. Since the blots for PLB were over-exposed (Figure 1A), some caution should be exercised while interpreting changes for PLB protein content. Treatment of control animals with prazosin showed no effect on SR protein expression (Figure 1). Since prazosin treatment did not affect the depressed SR Ca^{2+}-release and MF Ca^{2+}-stimulated ATPase activities in the infarcted animals, we did not attempt to determine their protein content by Western blotting. However, it is pointed out that the depression in SR Ca^{2+}-release activity has been demonstrated to be associated with a decrease in SR Ca^{2+}-release channel protein content whereas the depressed MF Ca^{2+}-stimulated ATPase activity was correlated with a shift in protein content of MHC isozymes [30,34] in the MI-induced heart failure.

Alteration in SR and MHC mRNA expression

The steady-state mRNA levels for the SR and MHC proteins were determined by Northern blot analysis (Figures 2,3). The MI rat hearts exhibited a decrease in the level of mRNA for SR proteins with 24% reduction in SERCA2a (Figure 2A), and 22% reduction in PLB (Figure 2B); however, there was no change in the level of mRNA for RyR (Figure 3B). The α- and β-MHC mRNA levels were also altered in the 20 wks MI hearts where α-MHC was decreased by 41% (Figure 3C), and β-MHC was increased by 170% (Figure 3D). Treatment of infarcted animals with prazosin did not show any significant changes in mRNA levels for either SR or MF proteins (Figures 2 and 3). Likewise, prazosin had no effect in these parameters for sham control hearts (Figures 2 and 3).

Discussion

We have shown the presence of cardiac dysfunction (as reflected by depressed LVSP, +dP/dt, -dP/dt, CO, EF, and FS, as well as increased LVEDP) and lung congestion (as reflected by increased lung wet wt/dry wt ratio) in infarcted animals due to coronary occlusion for a period of 20 wks. Since cardiac dysfunction in these infarcted animals was associated with cardiac hypertrophy (as indicated from increased heart wt/body wt ratio), increases in systolic and diastolic LV wall thickness and LV dimensions, and a decrease in IVSs, it is likely that heart failure in 20 wks MI animals was a consequence of cardiac remodeling. It should be pointed out that the progression of heart failure has been observed to be associated with cardiac dysfunction and

Parameter	Sham	MI	Sham + PRAZ	MI + PRAZ
A. **Sarcoplasmic reticulum activity:**				
Ca^{2+}-uptake (nmol Ca^{2+}/mg/min)	56.1 ± 2.10	16.8 ± 1.45*	50.2 ± 2.43	23.5 ± 1.12#
Ca^{2+}-release (nmol Ca^{2+}/mg/15 sec)	8.8 ± 0.45	4.0 ± 0.61*	8.7 ± 0.36	4.4 ± 0.52
B. **Myofibrillar ATPase activity**				
Mg^{2+}-ATPase (μmol Pi/mg/hr)	3.3 ± 0.11	3.5 ± 0.13	3.2 ± 0.15	3.6 ± 0.13
Ca^{2+}-stimulated ATPase (μmol Pi/mg/hr)	12.8 ± 0.55	8.0 ± 0.50*	80 ± 4.9	8.5 ± 0.61

Values are mean SE of 7 animals in each group. MI–Myocardial Infarction; PRAZ–Prazosin (10 mg/kg/day); *P<0.05 compared with the 20 week sham group; #P<0.05 compared with the 20 week MI group

Table 4: SR Ca^{2+}-transport and MF ATPase activities in sham and infarcted animals with and without prazosin treatment for 8 weeks starting at 12 weeks after coronary artery occlusion.

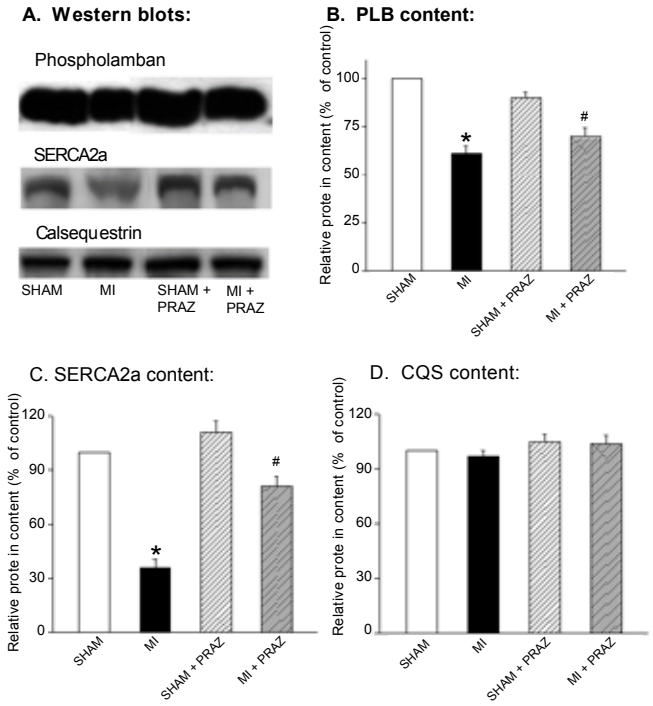

Figure 1: Typical Western blots (panel A) and relative protein content of sarcoplasmic reticular (SR) phospholamban (PLB, panel B), sarcoplasmic reticulum Ca^{2+}-pump ATPase (SERCA2a, panel C), and calsequestrin (CQS, panel D) from sham and infarcted (MI) rat hearts with or without treatment. Values are mean ± SE of 6 animals in each group. PRAZ - Prazosin; *P<0.05 compared with sham. #P<0.05 compared with MI group.

cardiac remodeling during 4 to 12 wks of inducing MI in rats [27,31,33-36]. Furthermore, cardiac remodeling is considered to be a major mechanism for the development of cardiac dysfunction in failing hearts [23-25]. It should also be noted that SR Ca^{2+}-transport activities and gene expression for SR proteins were found to be depressed whereas depression in MF Ca^{2+}-stimulated ATPase activity was associated with a shift in genes for MHC in 20 wks infarcted hearts. These observations are in agreement with earlier reports showing subcellular defects in heart failure due to MI in rats for 8 wks [30,33-36]. Since plasma levels of NE, EPI, and dopamine were markedly elevated in 20 wks infarcted animals and since the elevated levels of circulating catecholamines are known to produce cardiac dysfunction, cardiac remodeling, and subcellular defects [26], it appears that the observed cardiac abnormalities in failing hearts due to MI may be a consequence of a prolonged activation of the sympathetic nervous system. Different β-AR blockers have also been shown to depress plasma level of NE and dopamine without any changes in the plasma level of EPI in 8 wks infarcted hearts [27]. Because other neurohormonal systems such as renin-angiotensin system are also activated in heart failure [23-25], their contribution in the pathogenesis of cardiac dysfunction and remodeling cannot be ruled out. Since mRNA level for SR Ca^{2+}-release channel in 20 wks MI hearts was not different from that in control hearts and since mRNA level for Ca^{2+}-release channel was depressed in 8 wks MI hearts [30], it is likely that changes in mRNA level for Ca^{2+}-channel are biphasic because no change in gene expression for Ca^{2+}-release channel was observed in 40 wks MI hearts [37]. Likewise, gene expression for SL Na^{+}-Ca^{2+} exchanger was depressed in 8 wks MI hearts whereas this parameter was increased in 40 wks infarcted hearts [32,37].

Treatment of 12 wks infarcted animals with prazosin for a period of 8 wks reversed cardiac hypertrophy and all parameters for cardiac dysfunction partially. Lung congestion as well as some parameters for cardiac remodeling such as increases in LV wall thickness and LVSs was reversed fully whereas others such as increase in LVIDs were attenuated partially and increase in LVIDd was not altered upon treating MI rats with prazosin. These beneficial effects of prazosin therapy may not be entirely due to the blockade of α-ARs and associated signal transduction mechanisms in the myocardium because the sympathetic nervous system activity was also depressed as the increased plasma level of NE, unlike that for EPI or dopamine, was fully reversed by treatment of infarcted animals with prazosin. Partial reversal of MI-induced cardiac dysfunction by prazosin was also associated with partial reversal of depressed SR Ca^{2+}-uptake as well as of depressed protein content for SR Ca^{2+}-pump and PLB proteins. Since the depression in mRNA levels for SR Ca^{2+}-pump and PLB proteins were not altered by prazosin treatment, it is likely that the observed changes due to prazosin in SR Ca^{2+}-uptake process may occur at post-translational level rather than at the transcriptional level. This view is further attested by our observations that neither the depressed MF Ca^{2+}-stimulated ATPase activity and depressed gene expression for α-MHC, nor the increased mRNA level for β-MHC was modified by treatment of infarcted animals with prazosin.

The beneficial effects of prazosin on cardiac dysfunction, cardiac remodeling and defects in SR Ca^{2+}-uptake system may not be due to

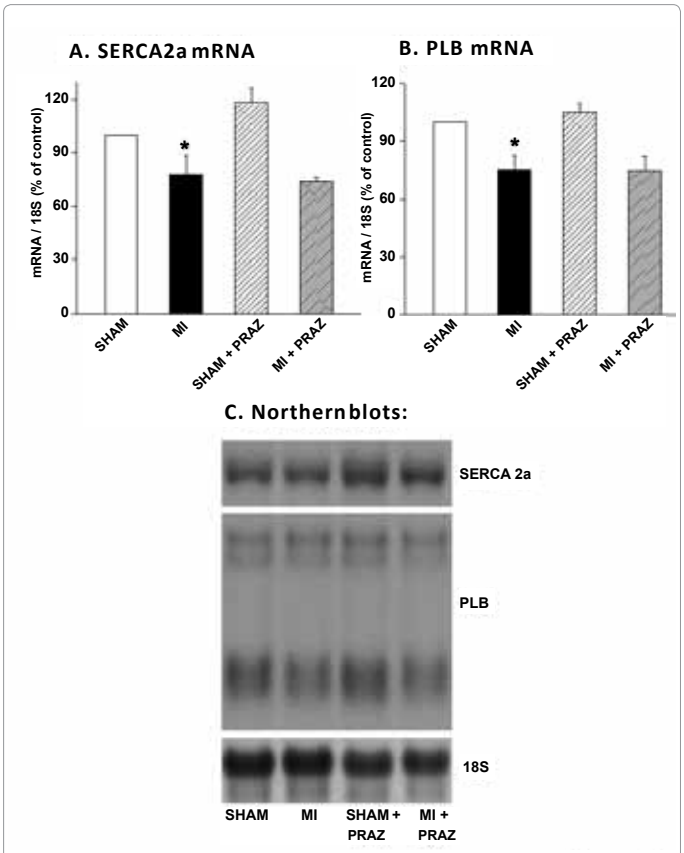

Figure 2: Relative mRNA content of sarcoplasmic reticular (SR) Ca^{2+}-pump ATPase (SERCA2a, panel A), phospholamban (PLB, panel B), and their corresponding Northern blots (panel C) from sham and infarcted (MI) rat hearts with or without treatment. Values are mean ± SE of 6 animals in each group. PRAZ - prazosin; *P<0.05 compared with sham.

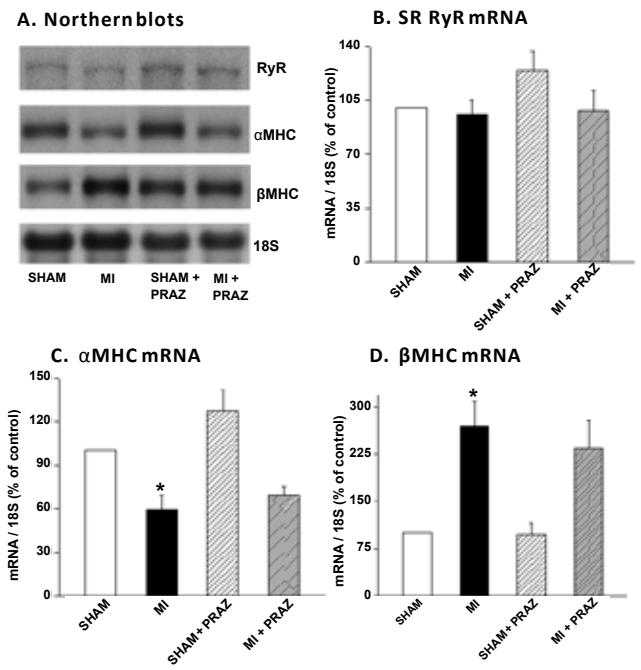

Figure 3: Typical Northern blots (panel A) and relative mRNA contents of sarcoplasmic reticular ryanodine receptor (RyR, panel B), alpha myosin heavy chain (α-MHC, panel C), and beta myosin heavy chain (β-MHC, panel D) isozymes from sham and infarcted (MI) rat hearts with or without treatment. Values are mean ± SE of 6 animals in each group. PRAZ - Prazosin; *P<0.05 compared with sham.

reduction of the infarct size or work overload on the heart because scar wt/LV wt ratio as well as heart rate in MI rats were not altered by treatment with prazosin. Although it is generally believed that the beneficial actions of α-AR blockers in heart failure are due to a decrease in the afterload [12-15], we did not observe any change in MAP in experimental rats upon prazosin treatment. This may be due to adjustment in blood pressure during 8 wks of prazosin administration or difference in drug responses in rats and humans upon chronic treatment. Furthermore, the observed effects of this drug on the infarcted heart are not due to any direct action of prazosin because it did not affect any parameters of cardiac dysfunction, cardiac remodeling or subcellular activity in the control animals. The results described in this study suggest that the beneficial effects of prazosin on cardiac dysfunction and cardiac remodeling as well as improvement of SR Ca^{2+}-pump activity in infarcted animals may be occurring due to lowering the elevated levels of plasma NE as well as blockade of α-AR in the heart. Nonetheless, our observations regarding the beneficial effects of α-AR blockade in heart failure do not support the view that stimulation of α-AR may serve as a mechanism for the maintenance of cardiac function during the development of heart failure [1,2,7,8]. On the contrary, our findings indicate that the activation of α-AR may contribute to the pathogenesis of cardiac dysfunction in heart failure and are in agreement with the role of α-AR suggested by others [11]. It is possible that the role of α-AR activation in the preservation of cardiac function or in the development of cardiac dysfunction may depend upon the stage of heart failure.

Although some clinical studies have shown the improvement of cardiac function upon treatment with α-AR blockers [12-15], others have failed to show any beneficial effects [20,21]. In spite of these controversial investigations, the results reported in this study provide experimental evidence that the use of α-AR blockers may prove to be of some value for the treatment of heart failure. However, exact molecular mechanisms, which can explain the beneficial effects of α-AR blockade in heart failure, are not understood. It is likely that attenuation of cardiac remodeling, activation of sympathetic nervous system and defects in SR Ca^{2+}-pump by α-AR blockers may play a critical role in improving cardiac function in heart failure. Such effects of α-AR blockers may be occurring at the level of both myocardium and sympathetic nerve terminals. In addition, the beneficial effects of α-AR blockers in heart failure appear to be related to their actions on coronary vasculature because the α-AR blockade has been demonstrated to improve coronary flow as well as myocardial metabolism and function in dogs with heart failure [19]. In view of the critical role of intracellular Ca^{2+}-overload in the development of heart failure due to MI [23-25], it is likely that α-AR blockers may attenuate the occurrence of intracellular Ca^{2+}-overload and thereby improve cardiac function. Since the stimulation of α-AR in cardimyocytes has been shown to activate phospholipase C, protein kinase C and extracellular signal-regulated kinases [38,39], it appears that the beneficial effects of α-AR blockers including prazosin may be elicited through the modification of this α-AR associated signal transduction mechanism. This view is consistent with earlier suggestions that phospholipid-mediated signaling plays an important role in the development of cardiac dysfunction in diseased myocardium and thus could serve as a noval target for the treatment of heart failure due to MI [40,41].

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