Reversal of Cardiac and Subcellular Remodeling in Congestive Heart Failure by Blockade of Catecholamine and Angiotensin Receptors

by

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A Thesis submitted to the Faculty of Graduate Studies of

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ABSTRACT

It is now well known that myocardial infarction (MI) is a leading cause of congestive heart failure (CHF) and cardiac remodeling is of paramount importance in both prevention and treatment in relation to cardiac dysfunction. The failing myocardium exhibits altered contractile properties, for which the underlying mechanisms involved in CHF due to MI remain to be elucidated. Activation of the sympathetic nervous system (SNS), in tandem with the renin angiotensin system (RAS), is a crucial element in the process of cardiac subcellular remodeling post MI. Although blockade of these systems has been shown to prevent cardiac remodeling and cardiac dysfunction associated with CHF due to MI, very little is known regarding the beneficial effects of the following agents for reversing the subcellular changes in the failing heart. Through utilization of the experimental model of rat coronary artery occlusion, the angiotensin II type 1 receptor (AT_1R) antagonist, losartan, the β-adrenergic receptor antagonist, metoprolol, and the α-adrenergic receptor antagonist, prazosin were individually employed to evaluate the possibility of reversing cardiac subcellular remodeling at the molecular and cellular levels. These drugs were initiated at 12 weeks post MI, which allowed the establishment of the cardiac subcellular remodeling and then the animals were used 8 weeks later. This study is unique in that it is one of the first ventures to attempt reversal of cardiac subcellular remodeling in already established CHF.

Since the sarcoplasmic reticulum (SR) is the main regulator of intracellular Ca^{2+} concentration in cardiac contraction and relaxation, we hypothesized that abnormalities in both the SR function and its regulation will contribute to cardiac contractile dysfunction in CHF due to MI. Accordingly, we examined overall cardiac performance, SR function,

SR molecular expression and plasma catecholamine levels in the failing rat heart at 20 wks following coronary occlusion. Additionally, these aspects were also evaluated after an 8 weeks treatment period with or without losartan (20 mg/kg/day), metoprolol (50 mg/kg/day), or prazosin (10 mg/kg/day) 12 weeks after coronary ligation. The remodeled myocardium possessed characteristics of fibrous scar tissue, and considerable cardiac hypertrophy was apparent from the increased heart weight and heart weight/body weight ratio. Further features of CHF were detected with the increased lung wet/dry weight ratio (typical of pulmonary congestion and edema), in addition to a weakened heart function, which was evident from the elevation of LVEDP and depression of $+dP/dt$ (LV pressure development), and $-dP/dt$ (LV pressure decay). Echocardiographic assessment of the infarcted animals indicated contractile dysfunction, as the ejection fraction (EF), fractional shortening (FS), and cardiac output (CO) were all significantly reduced in comparison to control hearts. M-Mode echocardiography further revealed that the internal cardiac structures of IVSs, LVIDd, LVPWs, and LVPWd were altered in the failing hearts.

In the isolated SR preparations, we established that the mRNA levels of phospolamban (PLB) and sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a) depicted profound alterations that corresponded to similar changes in the protein expression levels. These modifications were coupled to a decrease in cardiac SR Ca^{2+}-uptake activity by SERCA2a (the major Ca^{2+} handling protein in the heart), provided further disruption in Ca^{2+} homeostasis. On the other hand, there were no changes detected in the mRNA gene levels for the SR ryanodine receptor (RyR) or Ca^{2+}-release activity. It can therefore be postulated that the modifications in the subsequent mRNA levels of PLB and SERCA2a may play a critical role in the remodeling of the key SR protein levels. Supplementary

studies addressing the remodeling of myofibrils focused on the mRNA levels of the myosin heavy chain (MHC), which revealed a decrease in the α-MHC isozyme with a concomitant increase in the β-MHC isozyme. Measurement of myofibrillar Ca^{2+}-stimulated ATPase also showed a reduction in activity in the failing heart. This study revealed significantly elevated circulating levels of norepinephrine, epinephrine and dopamine in the infarcted animals as a result of the activation of the SNS in CHF due to MI.

Treatment of CHF animals with losartan, metoprolol, and prazosin significantly corrected lung edema, myocardial hypertrophy, and cardiac contractile dysfunction as measured by echocardiography and hemodynamics. Treatment of the infarcted animals with losartan, metoprolol and prazosin partially attenuated alterations in the expression of PLB and SERCA2a content. The gene expression for α- and β-MHC was only attenuated by losartan, and metoprolol could only attenuate the β-MHC mRNA. Moreover, there was no significant attenuation of any of the changes in the SERCA2a and PLB mRNA gene expression levels with any of the therapeutic agents. SR Ca^{2+}-uptake activities, along with alterations in the plasma catecholamine levels of norepinephrine were also partially reversed with the individual pharmacological treatment of losartan, metoprolol and prazosin, yet the dopamine levels were only affected by the losartan treatment. Our findings therefore suggest that partial reversal of cardiac hypertrophy, cardiac remodeling and cardiac dysfunction in CHF by losartan treatment may be due to remodeling of SR and myofibrillar organelles, where that by metoprolol and prazosin may be related to attenuation of SR Ca^{2+}-uptake, as well as SERCA2a and PLB content in the failing heart. Furthermore, the improvement in cardiac function due to metoprolol and prazosin may be

due to depression in the plasma NE levels, whereas that due to losartan and metoprolol may be related to reduction in the level of plasma dopamine.

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ANOVA	analysis of variance
ATP	adenosine triphosphate
bpm	beats per minute
BSA	bovine serum albumin
+dP/d*t*	rate of pressure development
-dP/d*t*	rate of pressure decay
α-MHC	α-myosin heavy chain
β-MHC	β-myosin heavy chain
ACE	angiotensin converting enzyme
Ang I	angiotensin I
Ang II	angiotensin II
ANP	atrial natriuretic peptide
AR	angiotensin receptor
AT_1R	angiotensin II type 1 receptor
AT_2R	angiotensin II type 2 receptor
BP	blood pressure
$[Ca^{2+}]_i$	intracellular concentration of free calcium
CAD	coronary artery disease
CHF	congestive heart failure
CO	cardiac output
CQS	calsequestrin
DAG	diacylglycerol

DCM	dilated cardiomyopathy
EDTA	ethylene glycol-bis(β-aminothyl ether) –N,N,N'-Tetra-acetic acid
EF	ejection fraction
FS	fractional shortening
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
HPLC	high performance liquid chromotography
HR	heart rate
HRP	horseradish peroxidase
IP_3	phosphatidylinositol-1,4,5-triphosphate
IVSDd	interventricular septal dimension (diastole)
IVSDs	interventricular septal dimension (systole)
kDa	kilo-dalton
LOS	losartan
LV	left ventricle
LVEDP	left ventricular end-diastolic pressure
LVIDd	left ventricular internal diameter (diastole)
LVIDs	left ventricular internal diameter (systole)
LVPWd	left ventricular posterior wall thickness (diastole)
LVPWs	left ventricular posterior wall thickness (systole)
LVSP	left ventriclular systolic pressure
MAP	mean arterial pressure
MET	metoprolol
MF	myofibrils
MHC	myosin heavy chain

MI	myocardial infarction
MLC	myosin light chain
MLCK	myosin light chain kinase
ND	not detected
PIP_2	phosphatidylinositol 4,5-bisphosphate
PLB	phospholamban
PRAZ	prazosin
PVDF	polyvinylidene diflouride
RAS	renin-angiotensin system
RV	right ventricle
RYR	ryanodine receptor
SERCA2a	sarcoplasmic reticulum Ca^{2+} ATPase
SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SL	sarcolemma
SNS	sympathethic nervous system
SR	sarcoplasmic reticulum
TBS	Tris-buffered saline
TBST	Tris-buffered saline with 0.1% Tween-20

I. LITERATURE REVIEW

1. Introduction

Congestive heart failure (CHF) is regarded as a major challenge for the health care system, as it encompasses high levels of morbidity and mortality (1). The causes of CHF include atherosclerosis, hypertension, diabetes, genetic cardiomyopathies and myocardial infarction (MI) (2). The lifetime risk of developing CHF is 1 in 5, where the long-term survival is relatively poor; up to one-third of those diagnosed with CHF die within the first 12 months, whereas half of the patients survive the five-year mark (3,4). It has also been reported that CHF currently affects more than five million Americans and is responsible for more than 700,000 deaths per year, costing the American economy US$50 billion annually (5). It is therefore disturbing that, with the universal aging of the population currently underway, CHF has become an escalating concern of epidemic proportion (6).

Due to these catastrophic statistics, it is imperative that the members of the biomedical community become aware as to how they can help improve therapy for different cardiac disorders. The inability of the myocardium to balance oxygen supply and oxygen demand, as well as to maintain efficient cardiac contractility during both rest and exercise is the hallmark of CHF, and in fact, it is the only cardiovascular problem that continues to increase in both incidence and prevalence (7). Due to the fact that various organs such as the heart, brain, liver, kidneys, lungs, skeletal muscle and blood vessels behave abnormally in CHF, in addition to the overload of fluid that drowns the peripheral organs in the body, the examination of the etiologies of CHF has become an

area of intensive research (8). Effectively, it should be noted that CHF is commonly associated with peripheral edema, lung congestion and/or liver enlargement. Particularly, CHF is the common concluding pathway of the majority for primary cardiovascular diseases such as hypertension, coronary atherosclerosis, cardiomyopathy, diabetes, myocarditis and congenital heart malformations (9). Furthermore, the progression of CHF emerges as a widespread and coordinated reaction to cardiac insult and injury, giving a complex cascade of events that underlie changes in the failing myocardium (10). As heart disease advances, the size of the heart increases, cardiac function begins to deteriorate and the symptoms of CHF become increasingly apparent (3). Specifically, cardiac remodeling is acknowledged as the prominent course of clinical CHF, and is defined as genome expression in association with molecular, cellular and interstitial alterations that are evident as the myocardium changes in size, shape and function upon experiencing pathophysiologic insult (3).

2. Cardiac Remodeling and CHF

CHF is invariably preceeded by cardiac hypertrophy which is an adaptive mechanism due to a wide variety of neurohormonal changes (6,11-14). Although cardiomyocyte hypertrophy is initially stimulated in response to mechanical alterations such as pressure or volume overload, various cytokines and hormones may also be involved in this process (15-17). An increase in the mechanical load occurs when the heart experiences an insult and the contractile elements of the myocardium are lost or rendered dysfunctional (10). Cardiac hypertrophy is a common reaction to the hemodynamic overload imposed on the heart (18,19) and most probably it is intended to promote efficient pumping by intensifying the number of cardiac contractile units, while

concomitantly decreasing the amount of wall stress by augmenting the wall thickness of the myocardium (10). Immediately following an ischemic episode such as an MI, there is an acute loss of myocardial cells that leads to uncharacteristic loading conditions, and results in dilatation of the ventricular chamber with a transformation in shape, to give a more spherical form (4). This reconstruction (cardiac remodeling) continues for several months, whereby the ultimate shape of the ventricle eventually becomes deleterious to the general functioning of the heart as a pump (4). It is believed that the progression of chamber enlargement due to MI is directly related to three factors: healing of the infarct, size of the infarct, and wall stress imposed on the ventricle (4,5). The phenomenon of cardiac remodeling is now a well established feature in the progression of cardiovascular disease and is currently prevailing as an important therapeutic target in the failing heart. Cardiac remodeling is generally associated with changes in genome expression, molecular mechanisms and cellular structures which become evident as a result of alterations in cardiac size, shape and function after serious injury to the heart (20). MI invariably leads to infarct expansion which can be defined as acute dilation and thinning of the area of infarction not explained by additional myocardial necrosis (21). The expansion of the infarct has been observed prior to and/or during the stage of necrotic tissue resorption before the massive deposition of collagen (22). Hence, infarct expansion is a model example of coupling amongst global changes in ventricular configuration and the subsequent principal cellular adaptations (23).

Although the mechanisms responsible for the transition of cardiac hypertrophy to heart failure (HF) have not yet been fully elucidated, remodeling of the ventricle as a result of marked alterations in the extracellular matrix has been proposed to be closely

associated with the advancement of HF (24-26). Key indications to support this view can be found in the extracellular space of the myocardium, which is home to a wide variety of cells that are structurally and functionally unique. Unlike the cardiomyocytes that comprise one-third of the cell population in the heart (27), endothelial cells (28), vascular smooth muscle cells (28), cardiac fibroblasts and macrophages reside in the cardiac interstitium and are collectively termed as non-myocyte cells (24). The growth of non-myocyte cells is referred to as interstitial structural remodeling and is usually accompanied by an accumulation of collagen (24). Due to the fact that non-myocyte and myocyte growth is independent of each other, hypertrophy of the myocardium can occur as a homogenous or heterogeneous process that is a result of proportionate or disproportionate non-myocyte growth, respectively (29,30). Adaptive hypertrophy occurs through the preservation of tissue homogeneity and proportionate non-myocyte growth, whereas maladaptive or pathological hypertrophy arises from the heterogeneity in myocardial structure and disproportionate non-myocyte cell growth (24). In addition to the alterations observed exterior to the cardiomyocyte, subcellular organelles such as sarcoplasmic reticulum (SR), sarcolemma (SL), mitochondria (MIT), and myofibrils (MF) in cardiomyocytes also become altered during the development of HF (8,18,31,32). The ability of the SR and SL to modulate Ca^{2+} and to regulate the contractile apparatus in cardiomyocytes (31-33) has provided a wealth of information to suggest that HF is indeed due to a defect in the ability of the SR and SL to manipulate Ca^{2+}. Changes in the composition of MF in HF are associated with alterations in the sensitivity of MF to Ca^{2+} (34-36). MIT abnormalities with respect to energy production at certain stages of CHF provide further evidence for the occurrence of subcellular defects/remodeling in HF

(8,32). However, the view regarding subcellular remodeling does not rule out the contribution of changes in the extracellular matrix to ventricular remodeling, but rather incorporates all systems into one network that compliment each other in the progression of cardiac hypertrophy into HF.

3. Ca^{2+} Homeostasis in CHF

Ca^{2+} is of paramount significance in cardiac physiology, as this cation is involved in processes as diverse as cell growth, metabolism, hormone secretion, motility, gene expression, protein trafficking, cell regulation, necrosis and apoptosis (37). The concentration gradient of Ca^{2+} in and out of the cell is criticial to its survival, where the intracellular cytoplasmic free Ca^{2+} concentration is 10^3-10^4 times less than that of the extracellular space (38). This large Ca^{2+} gradient is sustained through the involvement of the Ca^{2+} channel situated in the membrane networks of the cell surface and the inner SR (38). Upon electrical stimulus in the form of an action potential, the L-type voltage-gated Ca^{2+} channels in the SL membrane open to allow an influx of Ca^{2+} into the cytoplasmic space (39). This introduction of Ca^{2+} into the intracellular compartment gives rise to a rapid release of Ca^{2+} from the SR Ca^{2+}-stores through its ryanodine-sensitive Ca^{2+}-release channels, located in the area adjacent to the L-type Ca^{2+} channels (40-42). This entire process is referred to as Ca^{2+}-induced-Ca^{2+}-release (43). Immediately following the release of Ca^{2+} into the cytosol, inactivation of the L-type Ca^{2+} channel occurs, as Ca^{2+} binds to it on the cytosolic side, thereby contributing to a process called Ca^{2+}-dependent inactivation of Ca^{2+} influx (44,45). This intricate cycle is maintained in the cell to promote survival based on a local negative feedback effect, which starts with a large influx of Ca^{2+} that leads to release of Ca^{2+} from the SR to counteract more Ca^{2+} influx by

binding to the L-type channel on the cytosolic side, thereby inactivating the whole process (46).

In cardiac muscle, Ca^{2+} is the ubiquitous second messenger, which once released into the cytosol is the key component for initiating the transition of the resting state to the contractile state via binding to regulatory proteins in the contractile apparatus (43,47). When the concentration of free Ca^{2+} in the cytoplasm $[Ca^{2+}]_i$ is raised above the critical level, Ca^{2+} binds to troponin C (46). Troponin is a hetero-trimer that is composed of three distinct proteins: the calcium receptor (Tn-C), the inhibitor of the actin-myosin binding site (Tn-I) and the binding portion of troponin that effectively relays the Ca^{2+}-binding signal from Tn-C to the thin filament through the interaction of Tn-C, Tn-T and tropomyosin (47). The contractile machinery of the cell is turned on as a result of the association of Ca^{2+} with troponin C, but is quickly turned off for the relaxation process as Ca^{2+} dissociates from troponin C when the $[Ca^{2+}]_i$ is lowered (46).

Essentially, $[Ca^{2+}]_i$ is lowered mainly by the uptake of Ca^{2+} back into the SR via a Ca^{2+}-pump ATPase (SERCA2a) (48,49). In addition, $[Ca^{2+}]_i$ is lowered via: (a) Ca^{2+} exchange for external Na^+ by the SL Na^+/Ca^{2+} exchanger (48,50) (b) SL Ca^{2+}-pump that expels Ca^{2+} using ATP as an energy source (50), and (c) uptake of Ca^{2+} by the MIT (51). Though the SL Ca^{2+} pump and MIT participate in the uptake of Ca^{2+} during the contraction-relaxation cycle, the amount is minimal in comparison to other sites, and the mechanism of MIT uptake is not clearly understood (52). The Na^+/Ca^{2+} on the other hand, is driven by an electrochemical gradient, and essentially extrudes Ca^{2+} for exchange of Na^+ that is brought into the cell (53). It is interesting to note that the removal of Ca^{2+} from the cell during diastole is species specific. Different studies have shown that

rat ventricular myocardium utilizes SERCA2a to sequester 92% of the Ca^{2+} into the SR, while only 7% of Ca^{2+} is expelled by the Na^{+}/Ca^{2+} exchanger (54,55). However, in the human, rabbit, ferret, guinea pig and cat, it is observed that SERCA2a takes in 70-75% of the Ca^{2+} into the SR, leaving the remainder 25-30% to be removed by the Na^{+}/Ca^{2+} exchanger. The rat shows a higher SERCA2a activity in comparison to the rabbit, as it contains a greater concentration of protein pumps (56), whereas Ca^{2+}-movements in the mouse heart are quantitatively similar to those of the rat (57).

Despite the existence of a large amount of information concerning alterations in subcellular organelles in cardiac hypertrophy leading to HF, it is apparent that the mechanisms of subcellular remodeling remain poorly understood. In view of the fact that regulation of Ca^{2+} flow in and out of the cardiomyocyte is dependent upon the SL and the SR for efficient contraction and relaxation, there is a large interest concerning the abnormalities in the function of these specific cardiac membrane networks in CHF (58). Due to the fact that the regulation of Ca^{2+} movements in and out of the cardiac cell is directly associated with the SL membrane and the SR on a beat-to-beat basis, it is not a surprise that much attention is focused on the functional defects of these cardiac membrane systems at various stages of HF (4). It has been observed that about 80% of Ca^{2+} movement involved in the excitation-contraction process throughout the cell occurs via the SR, while the remaining, 20% of the Ca^{2+}, is transported across the SL by two transport systems known as the Na^{+}-Ca^{2+} exchanger and the plasma membrane Ca^{2+}-pump (59,60). An alteration in the cardiac cellular Ca^{2+} homeostasis can be attributed to irregular transmembrane movements of several cations that include Na^{+} and K^{+} (61). A major component of the SL is the Na^{+}-K^{+} ATPase, which was discovered in 1997 by

Skou (62,63), and is found in nearly all animal tissues, including the human myocardium (64). The Na^+-K^+ ATPase regulates the active transport of 3 Na^+ out of the cell, while transporting 2 K^+ ions into the cell (65). Furthermore, it is the most abundant protein found in the SL (66), and consists of an α-catalytic subunit and a glycosylated β subunit (67). The failing myocardium exhibits altered contractile properties, for which the underlying mechanisms involved in HF due to MI remain to be elucidated. Current propositions that are involved in addressing this issue include alterations in SL gene and protein expression, as well as functional changes of the SL proteins. Such alterations can be seen to result in molecular structure of the SL membrane with respect to its protein composition in the failing heart. Various proteins present in the SL membrane and their functions are shown in Figure 1. In addition, the major storage of Ca^{2+} in cardiomyocytes is the SR which is of crucial importance to the performance of the heart (68) and a wide variety of changes in the molecular structure of SR has been identified in the failing heart. Through prominent features of its ability to regulate the $[Ca^{2+}]_i$, the SR is the vital link in cardiac performance on a beat-to-beat basis (69). The SR has both longitudinal and junctional sections, which are found in the I-band of the sarcomere (70,71) and are home to many regulatory proteins that help the SR function as a whole (72). Included in the longitudinal SR is phospholamban (PLB) and SERCA2a whereas the ryanodine receptor (RyR) is located in the junctional SR (73). The sequence of events in cardiac contractility is regulated by the interaction amongst these specific proteins. Of primary interest, is the interaction between the SR protein PLB and SERCA2a. The involvement of different SR proteins in Ca^{2+}-release and Ca^{2+}-uptake in cardiomyocytes is depicted in Figure 1.

4. Modification of SL Function in CHF

A prominent member of the SL protein family is the Na^+-K^+ ATPase (62, 63), which exists in virtually all animal tissues, as well as the human myocardium (74). This enzyme is responsible for the active transport of Na^+ ions out of the cell, while simultaneously importing K^+ ions (65), and hence functions to maintain cell volume, establish ionic gradients and preserve membrane potential of the cardiomyocyte (75). Consequently, a defect in the function of this enzyme was found to be related to abnormalities in cardiac performance and has been outlined as a salient feature of the subcellular basis of CHF (75). A wide variety of SL changes including Na^+-K^+ ATPase were observed in several studies as shown in Table 1 (76-82).

The Na^+-K^+ ATPase is composed of different subunits such as α_1, α_2, α_3, β_1, β_2, and β_3, and several investigators have examined each subunit individually. Charlemagne *et al.* (65) reported that in mild and severe stages of hypertrophy, there was a reduction in the α_2 mRNA and protein levels, while the compensated stage of hypertrophy revealed no alterations in the α_1 and β_1 mRNA and protein levels. Furthermore, the α_3 mRNA and protein levels were increased at 5 days and 30-50 post-stenosis of the abdominal aorta, respectively. In another study, Ove Semb *et al.* (83) reported that 6 wks post-MI showed a reduction in α_2 mRNA and protein levels, with no alterations in the expression of the α_1 and β_1 subunits, but an increase in the α_3 subunit at the transcriptional level. This was supported by Book *et al.* (84), who reported that at 8 wks post-stenosis of the left renal artery, there was a decrease in the α_2 mRNA and protein levels with unchanged α_1 expression, and a reduction in the β_1 protein levels. A unique experimental animal models known as the UM-X7.1 cardiomyopathic hamster was studied by Kato *et al.* (85), who

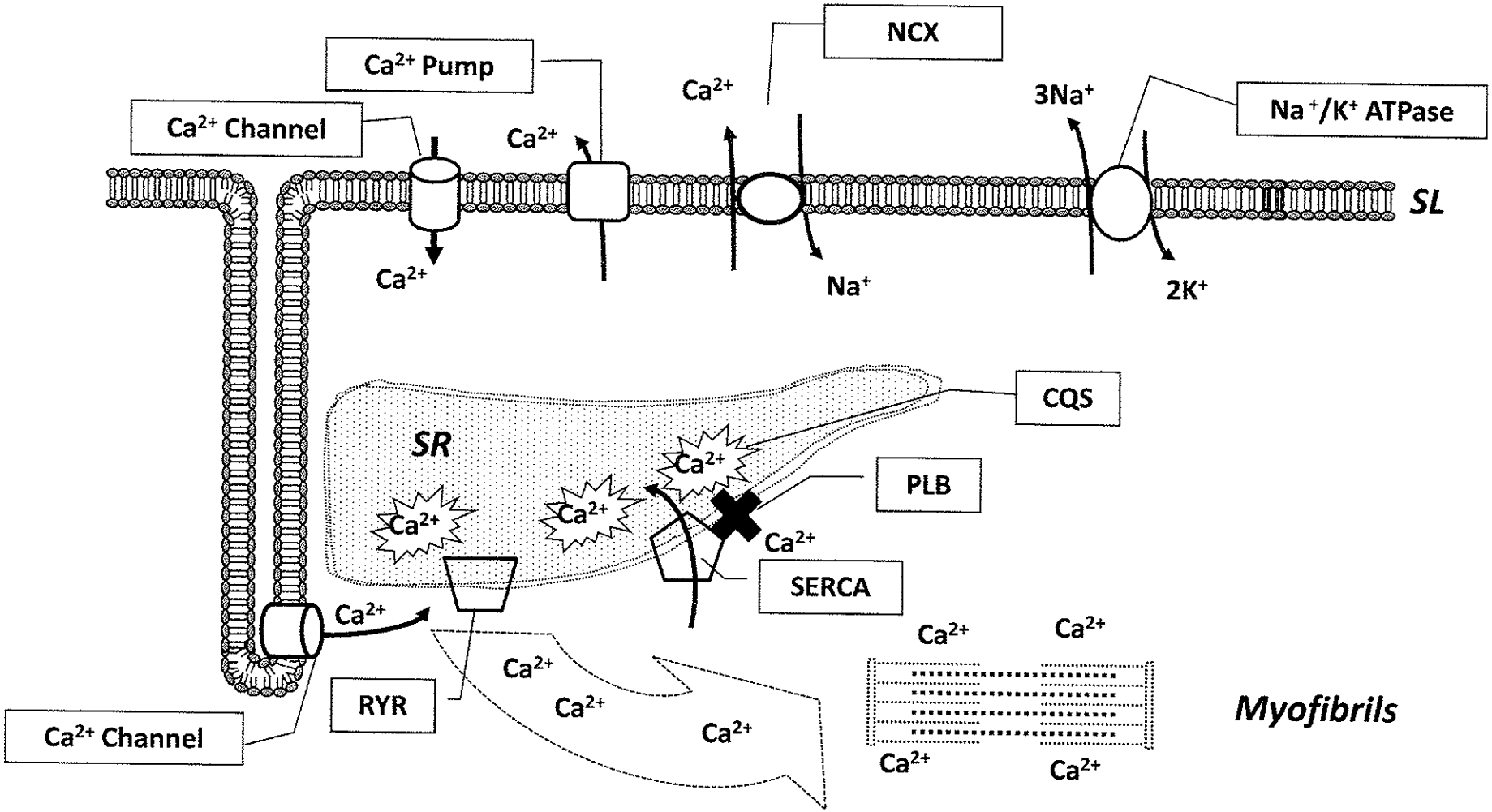


Figure 1. Calcium induced calcium release (CICR) in myocardial cells

SL - sarcolemma; SR - sarcoplasmic reticulum; RYR - ryanodine receptor; SERCA - sarcoplasmic reticulum Ca^{2+} pump; PLB - phospholamban; CQS – calsequestrin; NCX – Na^{+}/Ca^{2+} exchanger.

reported a decrease in the α_2 mRNA, protein and α_3 protein levels with enhanced α_1 and β_1 mRNA and protein levels, with contrasting undetected levels of α_3 mRNA.

In terms of the functional alterations in the Na^+-K^+ ATPase, Dixon *et al.* (86) monitored its activity at 4, 8, and 16 wks post-MI, and discovered that at 4 wks, the activity was unchanged, but at 8 and 16 wks the activity was significantly decreased. This finding suggested that the activity of the Na^+-K^+ ATPase may conceivably play a part in the adaptive mechanism of the heart that occurs during the development of CHF. Auxiliary studies by Shao *et al.* (77) demonstrated that reduced activity of this SL enzyme was coupled with the reduction in the expression of the α_1, α_2, and β_1 mRNA and protein levels, in addition to an elevation in the expression of the α_3 subunit. Shao *et al.* (77) further concluded that when imidapril (an angiotensin converting enzyme inhibitor (ACE)) was administered in animals with CHF, the Na^+-K^+ ATPase activity improved and the changes in the gene expression of the SL proteins were attenuated as a consequence of the blockade of the renin-angiotensin system (RAS). Further studies by Ren *et al.* (78) revealed that a 37 wks treatment of 3 wks post-MI with imidapril attenuated the reduction in the activity of the Na^+-K^+ ATPase, with paralleled trends in the expression of both the Na^+-K^+ ATPase and Na^+-Ca^{2+} exchanger.

The Na^+-Ca^{2+} exchange protein, which uses the influx of Na^+ to extrude intracellular Ca^{2+}, is a prominent member of the SL proteins and is situated in the T-tubules closest to the sites of Ca^{2+} release from the SR (87). The fully mature form of the Na^+- Ca^{2+} exchange exists as 120 kDa and is responsible for maintaining Ca^{2+} homeostasis in the cell (88). Since its discovery, the kinetic parameters of this enzyme suggest that this system can encompass rapid Ca^{2+} transport in and out of the myocardial

Table 1. **Modifications in SL Membrane Protein Gene Expression Post MI**

Animal Model /Time Point	Genes of Interest	Findings	Reference
Male S-D Rat, CL / 8 wks	Na^+/K^+ ATPase	↓ Na^+/K^+ α_2 *mRNA* and *protein* ↑ Na^+/K^+ α_3 *mRNA* and *protein*; NC Na^+/K^+ α_1 and β_1	Guo *et al.* [76]
Male S-D Rat, CL / 7 wks	NCX, Na^+/K^+ ATPase	↓ NCX *mRNA* and *protein* levels ↓ Na^+/K^+ $\alpha_{1,2}$ and β_3 *protein* levels ↑ Na^+/K^+ α_3 *protein* levels	Shao *et al.* [77]
Male S-D Rat, CL / 37 wks	NCX, Na^+/K^+ ATPase	↓ Na^+/K^+ α_2 *mRNA* levels ↑ Na^+/K^+ $\alpha_{1,2}$ *mRNA* levels ↑ NCX *mRNA* levels	Ren *et al.* [78]
Human End Stage Heart Failure	NCX	↑ NCX *protein* levels	Schillinger *et al.* [79]
Male Wistar Rat, CL / 110 days	NCX	↑ NCX *mRNA* and *protein* levels	Sjaastad *et al.* [80]
Male Wistar Rat, CL /4 wks and 12 wks	NCX	↑ NCX *mRNA* at 4 wks and 12 wks	Hanatani *et al.* [81]
Male Wistar Rat, CL / 1 wk, 3wks, 3 months	NCX	↑ NCX *mRNA* at 1 wk and 3 wks ↓ NCX *mRNA* at 3 months	Yoshiyama *et al.* [82]

S-D – Sprague Dawley; **CL** - coronary ligation; **MI** – myocardial infarction; **SHR** – spontaneously hypertensive rats; **NCX** – Na^+/Ca^{2+} exchanger; **Na^+/K^+** – Na^+/K^+ ATPase; **wks** – weeks; **NC** – no change observed.

cell during the cardiac contractile cycle (89). Though its significance in cardiac excitation-contraction coupling in normal heart has been well established, its role in the failing myocardium has yet to be fully characterized. Some of the studies indicating alterations in SL Na^+-Ca^{2+} exchange activity, as well as gene and protein expressions, are shown in Table 1. In a clinical study relating dilated cardiomyopathy (DCM) and coronary artery disease (CAD), Studer *et al.* (90) evaluated the expression of the Na^+-Ca^{2+} exchange together with the SR Ca^{2+} ATPase (SERCA). Amongst the two patient groups employed, the mRNA and protein levels of the Na^+-Ca^{2+} exchange were elevated in contrast to the reduction in the mRNA and protein levels of SERCA (90); this observation gave rise to the idea that the increased expression of the Na^+-Ca^{2+} exchange somewhat compensated for the diminished function of the SR to remove Ca^{2+} from the cytosol during relaxation. Furthermore, these findings were supplemented by Hasenfuss *et al.* (91) who affirmed that the decreased levels of SERCA in concert with unaltered levels of the Na^+-Ca^{2+}exchange, accounted for the disorder in diastolic dysfunction. On the other hand, early stages of HF due to MI have shown a reduction in the activity, mRNA and protein expression of Na^+-Ca^{2+} exchange (7,77). Schillinger *et al.* (79) illustrated, in the hours preceding cardiac transplantation, that the increase in the Na^+-Ca^{2+} exchange, collectively with the reduction in SERCA, provided a substantial association amongst neurohormonal levels of epinephrine and SL activity of the Na^+-Ca^{2+} exchange. They further projected that during CHF, the activation of the sympathetic nervous system (SNS) conceivably amplified the expression of the Na^+-Ca^{2+} exchange, which may have potentially had a role in the onset of malignant ventricular arrhythmias. The intensifying concern of progressing arrhythmias in CHF was previously examined by

Reinecke *et al.* (61). It was indicated that the enhanced activity of the Na^+-Ca^{2+} exchange in end-stage HF was a result of its increased protein levels and that this increase provided an augmented influx of Na^+, which was further associated with potential membrane depolarizations to create amplified arrhythmogenesis if the Na^+-Ca^{2+} sustained movement predominantly in the forward mode.

5. Modification of SR Function in CHF

During cardiac relaxation, Ca^{2+} is pumped from the cytosol into the SR through the 105 kDa SR Ca^{2+} ATPase pump (92, 93). As this enzyme is responsible for the diastolic phase of the cardiac cycle, any impairments in this process of Ca^{2+} sequestration could possibly contribute to the pathophysiology of HF. Supporting evidence for this postulation regarding cardiac contractile dysfunction in the failing heart include: a) an increased abundance of SERCA with contrasting decreased levels of PLB in response to prolonged exposure to thyroxine (94, 95); b) an abnormal force-frequency relationship, whereby increased frequency of stimulation gives a decreased developed tension (96); and c) a down-regulated β-adrenergic receptor to give rise to reduced levels of cAMP, which further affects the regulation of PLB and in turn, disrupts its negative inhibitory regulation of SERCA2a (97,98). Extensive research has been focused on elucidating the mechanisms involved in the transition of the myocardium to HF in various quantifiable experimental models. Clinical studies performed on the isolated human myocardium have shown an overall decrease in SERCA mRNA (99-101) and protein levels, in addition to a reduction in the abnormal handing of Ca^{2+} by SERCA, itself (102,103). To assess the validity of these applications, different investigators have attempted to compare the findings in animal models of HF with the failing human myocardium. In a report by

Movsesian *et al.* (104), there are findings that show a reduction in the Ca^{2+} sequestration of the failing human myocardium, consistent with those of the animal models, and can be attributed to decreased levels of SERCA mRNA produced during gene transcription. Further studies supporting this decrease in SERCA expression and function in the failing heart include models of the pressure-overloaded rat (93), the tachycardia-induced mongrel dog (105), the volume-overloaded rat (106), and the transgenically engineered hypertensive rat (107). Though this offers insight into the molecular pathogenesis of CHF, the data that is accumulated raises more questions than that which have been answered. If in fact, the protein level of SERCA remains unchanged in the failing heart, the answer may possibly lie in the complex mechanisms concerning transcription, translation and protein degradation as an entire cumulative process (104). Varying degrees of alterations in different SR protein functions in CHF due to MI are shown in Table 2 (108-120).

The Ca^{2+} uptake process of SERCA is intimately moderated through another SR protein, PLB. Composed of five equal monomers, this 30 kDa protein (121) inhibits SERCA through direct interaction which depresses the transport of Ca^{2+} into the SR (122). As phosphorylation of PLB relieves this inhibition and allows for Ca^{2+} uptake to occur, it is thought that this resulting de-inhibition through kinase activity is the principal molecular mechanism responsible for the inotropic effects involved in the β-adrenergic receptor system (104). Compelling evidence to substantiate this phenomenon was seen in the study involving the isoproterenol myocardial response in mice deficient in the PLB gene in comparison to their control (123). This study has demonstrated that the PLB-deficient mice exhibited high rates of contraction and relaxation in the absence of

isoproterenol with no increase in response in its presence, which correlated well with the fact that the control mice had low rates of contraction and relaxation in the absence of isoproterenol, but showed increased rates with progressive exposure to isoproterenol. In accordance, these results truly reflect the mechanism by which the phosphorylation of PLB removes the inhibitory effect on SERCA to permit the transport of Ca^{2+} into the SR.

Ca^{2+} release from the SR is achieved through the RyR, which is composed of four monomers of ~560,000 kDa (124,125). Evidently, the cardiac RyR mRNA is unique to the myocardium and is not expressed in fast- or slow-twitch skeletal muscle (126,127). Although the action of Ca^{2+} pumping into the SR has been extensively studied in CHF of humans and animal models, a small amount of literature is available on the Ca^{2+} release section of the Ca^{2+} cycle. When SR membrane vesicles were incorporated into artificial planar phospholipid bilayers and the activity of single channels was subsequently recorded using voltage clamp conditions, unaltered characteristic behaviour of these channels was observed in HF from ischemic cardiomyopathy, DCM, congenital disease or valvular disease as compared to normal hearts (128,129). Yet contradictory to these results, another study revealed that the threshold of Ca^{2+} release, as induced by caffeine, was remarkably increased in the hearts of patients with DCM as opposed to normal hearts, thus suggesting an impaired gating mechanism in the Ca^{2+}-release channel (130). In studies comparing the Ca^{2+}-release activity in both pressure-overloaded and volume-overloaded rats, Hisamatsu *et al.* (106) have documented enhanced Ca^{2+}-release in the compensated left ventricular hypertrophy stage of the pressure-overloaded model, with a contrasting decrease in Ca^{2+}-release activity and number of RyR in the volume-overloaded model. Cory *et al.* (131) have observed that both the density of the SR

Table 2. **Modifications in the SR Protein Gene Expression Post MI**

Animal Model /Time Point	Genes of Interest	Findings	Reference
Male S-D Rat, CL/ 5 wks	SERCA2a/PLB/RyR	↓SERCA2a/PLB/RyR *mRNA* ↓ SERCA2a/PLB/RyR *protein*	Sanganalmath *et al.* [108]
Male S-D Rat, CL/1day, 2 wks, 4 wks	SERCA2a	↓ SERCA2a *mRNA* Day 1 ↓ SERCA2a *protein* at 4 wks	Sallinen *et al.* [109]
Male Wistar Rat, CL/ 12 wks	SERCA2a	↓ SERCA2a *mRNA* levels	Prunier *et al.* [110]
Male S-D Rat, CL / 7 wks	SERCA2a/PLB/RyR	↓ SERCA2a/PLB/RyR *mRNA* levels	Shao *et al.* [77]
Male S-D Rat, CL / 37 wks	SERCA2a/PLB/RyR/CQS	*NC* SERCA2a/RyR/CQS *mRNA* ↓ PLB *mRNA* levels	Ren *et al.* [78]
Male S-D Rat, CL / 16 wks	SERCA2a/PLB/RyR	↓ SERCA2a, PLB *mRNA* levels ↑ RyR *mRNA* levels	Xu *et al.* [111]
Male S-D Rat, CL / 7 wks	SERCA2a/PLB/RYR/CQS	↓ SERCA2a/PLB/RyR/CQS *mRNA* ↓ SERCA2a/PLB/RyR/CQS *protein*	Guo *et al.* [112]
Human End Stage Heart Failure	SERCA2a	↓ SERCA2a *protein*	Schillinger *et al.* [79]
Male S-D Rat, CL / 12 wks	SERCA2a/RyR	↓ SERCA2a/RyR *mRNA* levels	Sakai *et al.* [113]

Table 2 cont'd.

Table 2 (Cont'd). **Modifications in the SR Protein Gene Expression Post MI**

Animal Model /Time Point	Genes of Interest	Findings	Reference
Male S-D Rat, CL / 6 wks	SERCA2a/PLB	*NC*	Ambrose *et al.* [114]
Human Heart DCM, ICM	SERCA2a	↓ SERCA2a *protein* levels	Hasenfuss *et al.* [91]
Male S-D Rat, CL / 8 wks	SERCA2a/PLB	↓ SERCA2a/PLB *mRNA & protein*	Shao *et al.* [115]
Male S-D Rat, CL / 3 wks	SERCA2a	↓ SERCA2a *protein* levels	Zhang *et al.* [116]
Male S-D Rat, CL/ 4, 12 wks	SERCA2a	↓ SERCA2a *mRNA* 12 wks	Hanatani *et al.* [81]
Male S-D Rat, CL / 4 wks	SERCA2a	↓ SERCA2a *mRNA* levels	Iijima *et al.* [117]
Human Idiopathic DCM	SERCA2a/PLB	*NC*	Munch *et al.* [118]
Male S-D Rat CL/ 1 day, 1 wk and 6 wks	SERCA2a/PLB	*NC* in SERCA2a *mRNA* or *protein* ↓ PLB *mRNA* transiently on Day1	Yue *et al.* [119]
Male S-D Rat, CL/ 4, 8 and 16 wks	SERCA2a	↓ SERCA2a *mRNA* at 4,8,16 wks ↓ SERCA2a *protein* at 8 & 16 wks	Zarain-Herzberg *et al.* [120]
Human DCM, CAD	SERCA2a	↓ SERCA2a *mRNA* and *protein*	Studer *et al.* [90]

S-D – Sprague Dawley; **CL** – coronary ligation; **MI** – myocardial infarction; **SERCA2a** – sarco(endo)plasmic reticulum Ca^{2+} ATPase; **PLB** – phospholamban; **RyR** – ryanodine receptor; **CQS** – calsequestrin; **DCM** – dilated cardiomyopathy; **ICM** – idiopathic cardiomyopathy; **CAD** – coronary artery disease; **NC** – no change observed; **weeks** – wks.

terminal cisternae and the activity of RyR were reduced in the Doberman Pinscher dog CHF model, as well as during rapid ventricular pacing in mongrel dogs. Additionally, Arai *et al.* (101) documented a reduction in RyR mRNA in patients suffering from end-stage HF from primary pulmonary hypertension, DCM, or ischemic heart disease. Furthermore, Pennock *et al.* (132) described that after ligation of the circumflex artery in New Zealand White rabbits, the administration of 3,5-diiodothyropropionic acid (DITPA) prevented the reduction in the protein density of RyR, with no measurable changes at the gene mRNA level, thereby improving SR function in the infarcted rabbit heart.

6. Modification of MF Proteins in CHF

The structural contractile unit of the myocardium controls the transition of the diastolic state to the activated state through various intricate steric, allosteric and cooperative mechanisms of the MF thick and thin filaments (133). Upon cardiac distress, the MF, which represent more than 50% of the cell volume, are considered to adapt by increasing in size, number and overall expression (134). One of the major components of the cardiac contractile apparatus is the myosin motor of the thick filament protein system, which acts as the cross-bridge for interacting with the thin filament system to produce force through the consumption of ATP (135). As shown in Table 3 (136-143), various studies dating back to the early 1960's have revealed a significant reduction in MF ATPase activity in the failing heart (144), including CHF due to mitral valve insufficiency, pressure-overload, idiopathic cardiomyopathy and CAD (145-149). Composed of two heavy chains, each associated with two different light chains (150,151), myosin is involved in the imperative process that influences cardiac systolic

and diastolic functions. The myosin light chains (MLC) are categorized into special groups and are called essential MLC (MLC-1) and regulatory MLC (MLC-2) (151). In contrast to the thick filament family, the troponin network is a collection of proteins that encompasses the thin filament regulatory elements (152) and plays a crucial role in Ca^{2+} sensitivity on the MF and regulating MF ATPase activity (153). Currently, it is believed that the binding and removal of Ca^{2+} from troponin transmits conformational changes to tropomyosin, which in due course activates the contractile elements for triggering the cross bridge action between the actin and myosin (154-158). Since the MF are considered as the contractile machinery in the cell, any structural or functional modifications to myosin, actin, troponin and tropomyosin may contribute to MF remodeling and thus, the pathogenesis of HF, which needs to be carefully evaluated.

Of particular importance, there are two genes located in tandem on chromosome 14 that encode the cardiac myosin heavy chain (MHC), and are termed α-MHC and β-MHC (159,160). Given that the α-MHC isoform results in a high-power, low economy ATPase activity, whereas the β-MHC isoform gives rise to a low-power, high economy ATPase myofilament activity, the events associated with cardiac stress promote a shift in expression toward the β-MHC for a more efficient performance (161-166). In a study by Swoap *et al.* (167), both systemic hypertension and caloric restriction resulted in the enhanced expression of β-MHC protein and mRNA levels due to increased transcription activity, in concert with a reduction in the expression of α-MHC protein and mRNA levels. Eble *et al.* (168) conducted another molecular study in the failing hearts of rabbits, and reported an increase in the MHC synthesis in left ventricular dysfunction due to chronic ventricular tachycardia that could be explained by an increased MHC

Table 3. **Modifications in MF Component Protein Gene Expression Post MI**

Animal Model /Time Point	Genes of Interest	Findings	Reference
Male S-D Rat, CL / 4 wks	α- and β-MHC	↓ α-MHC *mRNA*; ↑ β-MHC *mRNA*	Hart *et al.* [136]
Male S-D Rat, AB (PO) / 15 wks	α- and β-MHC	↓ α-MHC *mRNA*; ↑ β-MHC *mRNA*	Schwarzer *et al.* [137]
Male S-D Rat, CL / 5 wks	α- and β-MHC	↓ α-MHC *mRNA*; ↑ β-MHC *mRNA* ↓ α-MHC *protein*; ↑ β-MHC *protein*	Sanganalmath *et al.* [108]
Male S-D Rat, ACS (VO) / 4,10 wks	α-/β-MHC/ α-cardiac/α-SK	↑ β-MHC *mRNA* at 4 and10 wks *NC* α-MHC/ α-cardiac/α-SK 4 wks ↓ α-MHC *mRNA* at 10 wks *NC* α-cardiac/α-SK at 10 wks	Freire *et al* [138]
Male S-D Rat, CL / 8 wks	α- and β-MHC	↓ α-MHC *mRNA*; ↑ β-MHC *mRNA*	Wang *et al.* [139]
Male S-D Rat, CL / 7 wks	α- and β-MHC	↓ α-MHC *mRNA*; ↑ β-MHC *mRNA*	Wang *et al.* [140]
Male Wistar Rat, AB (PO) / 18 wks	α- and β-MHC	↓ α-MHC *mRNA*; ↑ β-MHC *mRNA*	Huang *et al.* [141]
Male S-D Rat / 1 day, 1 wk, 6 wks	β-MHC/α-SK	↑ β-MHC *mRNA* 1 day/1 wk/ 6 wks ↑ α-SK *mRNA* 1 day/1 wk/ 6 wks	Yue *et al.* [119]
Male S-D Rat, AB (PO) / 22 wks	α- and β-MHC	↓ α-MHC *protein*; ↑ β-MHC *protein*	Chang *et al.* [142]

Table 3 cont'd.

Table 3 (Cont'd). **Modifications in MF Component Protein Gene Expression Post MI**

Animal Model /Time Point	Genes of Interest	Findings	Reference
Male Wistar Rat, CL / 1 wk, 3 wks and 3 months	α-/β- MHC/ α-cardiac/α-SK ↑ β-MHC/α-SK *mRNA* levels *NC* α-cardiac *mRNA* levels	↓ α-MHC *mRNA* levels	Yoshiyama *et al.* [82]
Male S-D Rat, CL / 20 wks	α-SK	↓ α-SK *mRNA* levels	Simonini *et al.* [143]

S-D – Sprague Dawley; **CL** – coronary ligation; **MI** – myocardial infarction; **MHC** – myosin heavy chain; **α-cardiac** – α-actin cardiac; **α-SK** – α actin skeleton; **ACS** – aortocaval shunt; **AB** – aortic banding; **PO** – pressure overload; **SHR** – spontaneously hypertensive rat; **VO** – volume overload; **NC** – no change observed; **wks** – weeks.

translational efficiency. This response was further supported by Imamura *et al.* (169), who reported an elevated synthesis in MHC in dogs subjected to pressure-overload. Furthermore, in the rat model of pressure-overload hypertrophy, Toffolo *et al.* (170) described an augmentation in cell size followed by a change in the expression of myosin, to produce the slow migrating, economic V_3 isoform, while exhibiting an increased number of myofibril units during the adaptive process of the myocardium.

7. SNS and the Cardiovascular System

The ability of the heart to markedly increase contractility and heart rate during periods of stress is facilitated by the SNS that innervates the myocardium (171). To date, two different types of adrenergic receptors have been identified, namely the α-adrenergic and the β-adrenergic receptors (172); the α_1, β_1 and β_2 subtypes are expressed in the human heart (173-178). Described as a transmembrane signaling system situated in the SL membrane, the G-protein family are neighbors to the α- and β-adrenoceptors that couple different receptors via effector enzymes (179-181). Upon stimulation of the SNS, the stimulatory G-protein couples the β-adrenergic receptors with the activation of adenylyl cyclase to generate the second messenger cAMP, which further activates the cAMP-dependent protein kinase to subsequently phosphorylate the SR protein PLB, the slow inward Ca^{2+} channel, troponin I and the trans-acting regulatory proteins of the nucleus (171). This effect on slow inward Ca^{2+} channel promotes the entry of Ca^{2+} in cardiomyocytes, whereas the phosphorylation of PLB enhances the uptake of Ca^{2+} in the SR; these events result in increasing the rates of cardiac contraction and relaxation, and thus form the molecular basis for the action of catecholamines on the heart (Figure 2).

The cellular changes of the cardiomyocytes that are observed in the failing heart include cell hypertrophy, irregularities in Ca^{2+} homeostasis, cross-bridge cycling, electrical excitation-contraction coupling, as well as several alterations in the cytoskeletal framework of the cardiac cell. Recent reports indicate that a variety of these alterations are observed during the early stages of heart failure, while remainder of the changes are developed in the overt decompensated stages of the failing heart and can be linked with abnormalities in both systolic and diastolic contractile functioning (182). The SNS plays a vital role in regulating the cardiovascular system in both the healthy and diseased states (183-187), as it not only provides appropriate vascular resistance through arteriolar constriction, but also employs a sympathetic discharge on the heart to give enhanced chronotropic and intropic effects. Long term blood pressure (BP) in the closed vasculature is also maintained by regulation of the renin angiotensin system in the kidney for blood vessel growth and permeability (188). During regular physiological conditions, these mechanisms act in harmony to regulate the trio of cardiac, vascular and renal entities to uphold adequately functioning blood volume/pressure, perfusion pressure, CO and suitable blood distribution (189). However, in the event of the disruption of a healthy functioning myocardium that illustrates a decrease in CO and subsequent blood supply to end organs, it is the action of neurohormonal agents that are greatly amplified to provide a compensatory response to promptly support and restore essential circulatory function in order to maintain survival of the existing tissue and organs (189).

Activation of the β-adrenergic receptors by epinephrine and norepinephrine in response to myocardial ischemic insult acts to augment CO, which helps to maintain BP at an appropriate level. Simultaneous activation of α-adrenergic receptors by the same

hormones increase total peripheral resistance while shunting blood flow to organs of priority that include the heart and the brain (190). The increase in CO initially is compensatory, however, over time it places an increased workload and oxygen demand on the heart. In the same way, an increased peripheral resistance initially is beneficial for maintaining BP, but its enhanced afterload effect possesses an increased resistance against which the heart must pump (191-193). These effects are a result of the stimulation of the sympatho-humoral system that is originally focused to attenuate the ongoing systemic hypoperfusion, but eventually exacerbates the development of cardiac contractile dysfunction that in turn enhances extra-cardiac irregularities to form a positive feedback of sequence of events that act continuously in a vicious cycle to produce grim circumstances (194).

Notwithstanding the fact that the SNS offers a means of supporting cardiac contractile function, it has been documented that the failing human heart subjected to idiopathic DCM becomes less sensitive to stimulation of the adrenergic system (98,195). This observation can be partly explained by a selective loss of β_1-receptors that contribute to an overall reduction in the ratio of β_1/β_2 ratio, which ultimately gives rise to a decrease from the 80:20 ratio in the normal healthy heart to the approximate 60:40 ratio in the failing myocardium (196). In addition, the relative loss in β_1-receptors is proportional to the degree of HF (197) and it has been suggested that chronic stimulation of the adrenergic system accounts for the decrease in the steady state levels of β_1-adrenergic receptor mRNA (198, 199). Over-stimulation of the SNS is also accompanied by other biochemical alterations including an increase in the expression of the α-subunit of inhibitory G-proteins, as well as attenuation of the positive inotropic effect of the β-

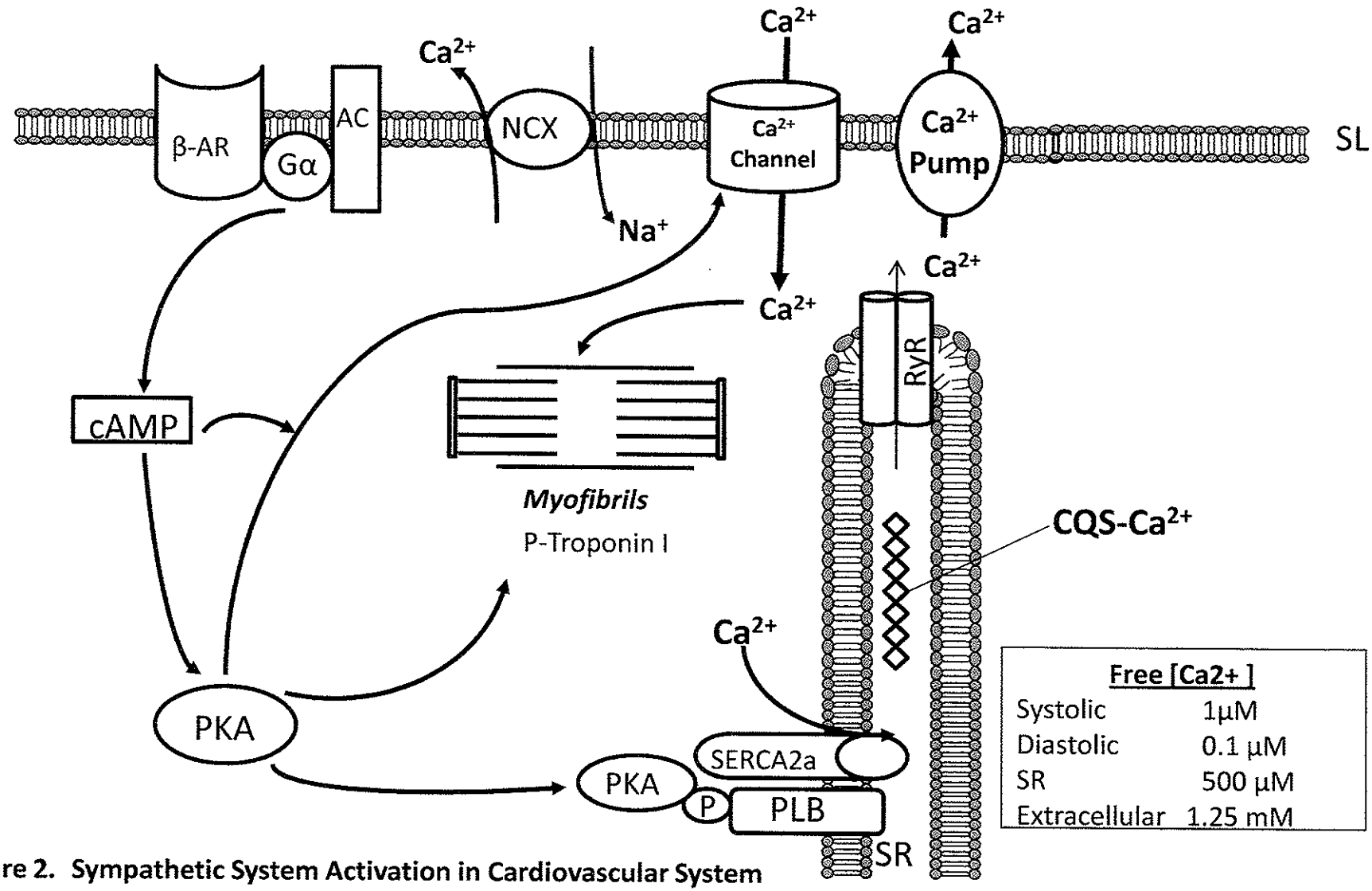


Figure 2. Sympathetic System Activation in Cardiovascular System

SL - sarcolemma; SR - sarcoplasmic reticulum; RYR - ryanodine receptor; SERCA - sarcoplasmic reticulum Ca^{2+} pump; PLB - phospholamban; CQS - calsequestrin; β-AR - β-adrenergic receptor; Gα - G-coupled protein; AC - adenylyl cyclase; NCX - Na^{+}/Ca^{2+} exchanger; PKA - protein kinase A; P- Phosphorylated; cAMP - cyclic AMP

adrenoceptor activation (200,201). With respect to cardiac performance in conjunction with SR function, Stein *et al.* (202) have reported that chronic stimulation of the β-adrenoceptor presented enhanced relaxation of the papillary muscles, and a reduction in the expression of protein levels of PLB and SERCA, where the regulatory phosphorylation of PLB was also reduced. This was additionally supported by Linck *et al.* (203) who showed that the administration of the β-adrenoceptor agonist, isoproterenol, gave rise to transiently increased relaxation due to an increase in the Ca^{2+} uptake activity with consequent decreases in mRNA and protein levels of both PLB and SERCA. Isoproterenol has also been reported to increase heart weight (204,205) and it has been shown that a strong negative correlation exists between catecholamine levels and life expectancy (203). Lai *et al.* (206) have documented that norepinephrine infusion in dog hearts produced a decrease in the mRNA and protein levels of SERCA, which paralleled those hearts of pacing-induced HF, with no change in the RyR, calsequestrin (CQS) and PLB mRNA levels.

8. Blockade of the SNS in MI

In the past, β-adrenergic receptor antagonists have been utilized for the remedy of several cardiovascular disorders including hypertension, angina pectoris, and MI (207). More specifically, the advantageous aspects of β-blockade on reducing mortality associated with MI have been greatly acknowledged for a period of more than ten years (208), whereby these benefits are observed especially in conditions of left ventricular dysfunction. Conventionally, the use of β-antagonists has been considered a contraindication in CHF, primarily because of their characteristic negative inotropic effects, yet recent data clearly indicate that β-blockade significantly decreased mortality

and improved the left ventricular function (208,209). As chronic heart failure progresses, myocardial contractile dysfunction occurs in response to the activation of the RAS and SNS, which inevitably releases various neurohormones to accelerate the downward slope of ventricular failure (210). Although the initial stages of the SNS activation are considered as compensatory, it is the deleterious effects of the advanced stages produced by the excess of catecholamines that are detrimental, and in fact they directly relate to the severity of the disease process (211). Moreover, it has been shown that increased levels of serum epinephrine are paralleled with the worst prognosis (212). In view of these intriguing reports, it has been previously proposed that blockade of the SNS in terms of the β-adrenergic receptors, may provide useful results in dampening the detrimental mechanisms triggered in myocardial injury (209). This can be achieved with long term therapy that consists of an initial low dosage of β-blockers that is titrated slowly and gradually over the course of treatment (190).

Since the initial sweep of Swedish studies that took place in the mid-1970's (213), there have been a substantial number of placebo-controlled clinical trials that have verified the advantageous outcomes of β-blockers (Table 4, 214-219). Particularly, single-centre studies have continuously validated the usefulness of these compounds in chronic heart failure by showing improvements in ejection fraction as well as in overall clinical status in subjects who are symptomatic with this disease (220-225). Beneficial outcomes of reduced morbidity and mortality were also apparent with the work of CIBIS-II with the drug bisoprolol (217), MERIT-HF with the drug metoprolol (216), and COPERNICUS with the drug carvedilol (226). Now that β-blockers have proven to be of ultimate prognostic value in the long term therapy of CHF, it is vastly accepted by the

current medical community as a customary pharmacological remedy in subjects who continue to be symptomatic regardless of therapy with other medications such as ACE inhibitors and diuretics (213). Several studies in experimental animals have also indicated the usefulness of β-adrenoceptor blocking agents in attenuating the adverse effects of MI (Table 5, 227-233).

9. RAS and the Cardiovascular System

The renin-angiotensin system (RAS) is a crucial element that plays a role in both the short- and long- term regulation of total body fluid, blood volume, overall electrolyte balance, arterial BP, as well as mediation of disease pathophysiology (234,235). Particularly, the RAS is intimately involved in the regualtion of cardiovascular function and structural remodeling, as its stimulation starts the pathway to deterioration of endothelial cell function, augmentation of growth, advancement of apoptosis, and the development of oxidative stress (236). Irrespective of the etiology of CHF, major players stimulated in the development of CHF include the neurohormonal and cytokine systems (237-239). Accordingly, the activation of the neurohormonal system occurs in a stepwise fashion, and is regarded as organ specific (203). The detrimental cycle of CHF is initiated by a decrease in CO that leads to the activation of a variety of vasoconstrictor neurohormonal compounds, in addition to Na^+ and water retention (241,242). It has been well established that the most studied neurohormonal systems are the SNS and RAS (243). Though these two complementary mechanistic pathways are required for optimal cardiac function, the result of cardiac injury causes these systems to pose a paradoxical degeneration in overall cardiac performance primarily through an increase in both preload and afterload (244). The RAS is a critical entity in the regulation of

cardiovascular function and structural remodeling in terms of everyday survival, as it acts primarily on the angiotensin II type 1 (AT_1) receptor in the myocardium (245). Accordingly, it has been shown by Ju *et al.* (246), that the presence of increased angiotensin II (Ang II) induces alterations in the mRNA levels of certain Ca^{2+} transport proteins and the increased levels of mRNA for the Na^{+}-Ca^{2+} exchange, the SR ryanodine Ca^{2+} receptor, and the SR SERCA protein, were attenuated upon treatment with the Ang II receptor antagonist, losartan. The effects of Ang II are further reported in a study by Rouet-Benzineb *et al.* (247), who observed that the transcription factor, NF-κB, was translocated into the nucleus from the cytoplasm via the protein kinase C (PKC) pathway in neonatal rat cardiomyocytes subjected to Ang II stimulation and this process was blocked with the administration of calphostatin C, a specific PKC inhibitor.

Throughout years of research, extensive efforts have been made to improve heart function in the infarcted animals upon treatments with various pharmacological interventions. Certain therapies include angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs). ARBs are a moderately new family of drugs that target diabetic nephropathy, HF, and hypertension to produce physiological effects similar to those of ACEIs (248). ACEI are directed against the active site of ACE, whereby their beneficial effects are viewed through their ability to suppress Ang II formation, in addition to exerting arteriolar dilation, venodilation and diuretic effects

Table 4. **Clinical Trials of β-Blockade in Patients with MI**

Clinical Trial	Drug Name	Human Dose	Study Highlights
BHAT [214]	Propanolol	180 mg/day 240 mg/day	Significant ↓ in overall mortality.
ISIS-1 [215]	Atenolol	100 mg/day	↓ overall vascular mortality at 1 year with ↓ risk of recurrent reinfarction, arrest and death from day 1-7.
MERIT-HF [216]	Metoprolol	200 mg/day	Metoprolol CR/XL with standard optimum therapy enhanced survival and was well tolerated.
CIBIS-II [217]	Bisoprolol	10 mg/day	Survival benefits in patients with stable HF only.
CAPRICORN [218]	Carvedilol	25 mg/twice daily	↓ both all cause and CV mortality + recurrent non-fatal MI in post acute MI with LV systolic dysfunction.
COPERNICUS [219]	Carvedilol	25 mg/twice daily	Alleviates severity of HF and ↓ risk of deterioration, hospitalization in euvolemic symptomatic patients at rest.

BHAT – β Blocker Heart Attack Trial; **ISIS-II** – First International Study of Infarct Survival Collaborative Group; **MERIT-HF** – Metoprolol CR/XL Randomized Intervention Trial in Congestive Heart Failure; **CIBIS-II** – Cardiac Insufficiency Bisoprolol Study II; **CAPRICORN** – Carvedilol Post Infarction Survival Control in Left Ventricular Dysfunction; **COPERNICUS** – Carvedilol Prospective Randomized Cumulative Survival Study; **MI** – myocardial infarction ; **LV** – left ventricular; **CV** – cardiovascular.

Table 5. **β_1-adrenergic Antagonists in Experimental MI Animal Models**

Animal Model	Drug	Dose	Therapeutic Effects	Reference
Male S-D Rats AB	Propranolol	40 mg/kg/day	↓ LV hypertrophy, dilation and lung congestion, but no significant improvement on survival.	Perlini *et al.* [227]
Male Wistar Rats CL	Metoprolol	250 mg/kg/day	↓ EF deterioration, post wall stress, LV dilation. ↑ Ca^{2+} transient amplitude, ↓ NCX activity, Ca^{2+} sensitivity.	Maczewski *et al.* [228]
Male S-D Rats CL	Carvedilol Metoprolol	30 mg/kg/day 60 mg/kg/day	Improved LV+ RV wt and SERCA2a mRNA levels Same effects as for carvedilol, but more significant improvement on SERCA2a mRNA expression.	Sun *et al.* [229]
Male S-D Rats CL	Metoprolol	1 mg/kg/h	Improved LV dimensions and volumes as well as ↓plasma levels IL-6, but ↑IL-1β. *NC* PCr/ATP.	Omerovic *et al.* [230]
Male S-D Rats CL	Metoprolol	5 mg/kg/h	Normalization of phosphocreatine/ATP, ↑SV & EF. ↓ plasma NE and brain natriuretic peptide.	Omerovic *et al.* [230]
Male S-D Rats CL	Metoprolol	10 mg/kg/day	Attenuated LVEDP, ↓ collagen deposition, but did not affect plasma NE or urinary NE excretion.	Latini *et al.* [232]
Male S-D Rats (CL)	Carvedilol	1 mg/kg	Drug that ↓ infarct size by ~50%	Feuerstein *et al.* [233]

S-D – Sprague Dawley; **CL** – coronary ligation; **MI** – myocardial infarction; **AB** – aortic banding; **LV** – left ventricle; **RV** – right ventricle; **EF** – ejection fraction; **SV** – stroke volume; **LVEDP** – left ventricular end diastolic pressure; **NCX** – Na^+/Ca^{2+} Exchange; **NE** – norepinephrine; **PCr/ATP** – phosphocreatine/ATP ratio; **NC** – no change observed.

(249). Various steps involved in the activation of RAS are given in Figure 3. In a long-term study focusing on the effects of the ACEI captopril, on left ventricular remodeling of the myocardial infarcted canine model, Jugdutt *et al.* (250) revealed attenuation of early infarct expansion, the absence of late wall thinning, a reduction in the diastolic bulging, and eradication of aneurysms, and a general restitution in overall cardiac systolic function, in addition to a marked reduction in preload and afterload. In another study involving ACEI treatment, McDonald *et al.* (251) discovered that during progressive ventricular remodeling in the myocardial infarcted rat, late captopril therapy attenuated further increase in cell length, which is associated with myocyte hypertrophy and growth of the cardiac interstitium due to MI. Thus, a study by Dixon *et al.* (252) that focused on the effects of the combination treatment of ramipril and losartan on collagen expression in the MI rat model, found that the administration of both the ACEI and AT_1R antagonist showed an overall reduction in cardiac fibrosis, which hypothesized that Ang II may be involved in the regulation of cardiac collagen synthesis after MI at the post-transcriptional site.

10. Blockade of RAS in MI

Upregulation of the RAS is believed to be associated with the development of various cardiovascular pathologies that include atherosclerosis, hypertension, cardiac hypertrophy, heart failure and neuropathies. Ang II, to some extent, is also produced by an ACE-independent pathway (253) and hence, ACEIs cannot completely block its production. Accordingly, ARBs, which are non-peptide competitive Ang II receptor antagonists (Figure 2), were developed to prevent specifically the binding of Ang II to the AT_1 receptor, thereby completely blocking the effect of the hormone. It has not yet been

determined if the overwhelming receptor blockade of ARBs is due to a slow dissociation kinetics of Ang II from its receptor, from an internalization of the ARB-induced receptor, or to alternative binding sites on the AT_1 receptor (254). Supposedly, this prolonged receptor blockade can be beneficial in situations of increased levels of the endogenous ligand or in situations of missed doses in therapeutic drug treatment. Furthermore, ARBs have been anticipated to act superiorly to ACEIs. ARBs are a family of the drugs with evolving indications in hypertension, HF and diabetic nephropathy, as their physiological effects are similar to those of ACEIs (255). By blocking the actions of Ang II at the receptor level in blood vessels, adrenals and in tissues, these agents are able to induce dilation of arterioles and veins, to prevent pathological changes in cardiac structure, to reduce the release of aldosterone, to increase renal Na^+ and water excretion, to reduce plasma volume, and to decrease cellular hypertrophy. Several studies in experimental animals with MI have shown that ARBs produce beneficial effects in improving cardiac function (Table 6, 256-270). Clinically ARBs are known to enhance LVEF, improve HF symptoms, increase exercise tolerance, and boost quality of life, in addition to reducing mortality and hospitalization (Table 7, 271-279). However, the indication of ARBs in HF was greatly ambiguous until the CHARM trials were published (255,277,278). This study specifically established the value of these drugs in systolic HF. In addition to CHARM, the initial trial of ELITE was also uniquely designed to compare the nephrotoxicity of the ARB losartan with the ACEI captopril in the elderly with HF. The result showed a significant survival advantage in favor of the ARB (271,273,278). However, the short-term study by Lang *et al.* (280) and the follow up study of ELITE II (281), which was adequately powered to assess survival in a similar patient group, failed to confirm the

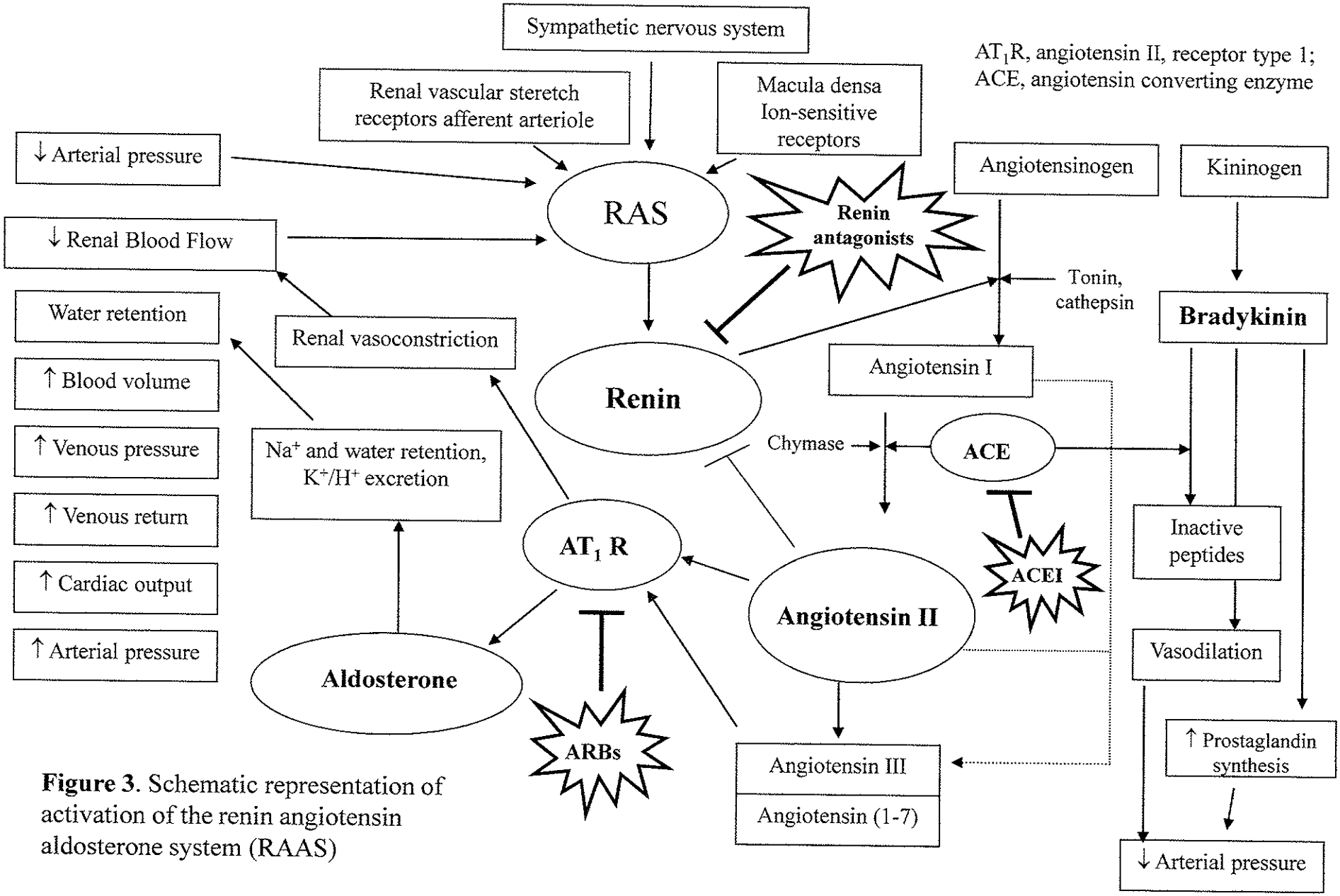


Figure 3. Schematic representation of activation of the renin angiotensin aldosterone system (RAAS)

superiority of losartan. In fact, it was observed that the ACEI reduced the onset of sudden death better than did the ARB. Meta-analysis of ARBs in CHF and high-risk MI revealed that ACEIs and ARBs do not differ in efficacy for reducing all-cause mortality and hospitalization for HF (281,282,283), and therefore, ARBs are suitable alternatives to ACEIs in CHF or high-risk MI. Future works include an additional ongoing trial termed I-PRESERVE, which attempts to test the effects of ARBs in HF with preserved systolic function (282). The concern of using ARBs in combination with ACEIs still remains vague, and continues to pose a challenge to both clinicians and scientists. In some trials, the addition of ARBs to an ACEI did not provide any additional benefit in combating CHF (272,276,284).

Based on this finding, ARBs should not be routinely added to the otherwise stable HF patient receiving a combination of an ACEI and a β-blocker. Nevertheless, the addition of ARBs can be beneficial in terms of reducing hospitalization for HF and improving the quality of life (271,285-287), with favorable effects on hemodynamics, ventricular remodeling, and neurohormonal profile (285). The anti-ischemic effect of ARBs was more than that of Ca^{2+}-channel blockers (275), yet similar to that observed with ACEIs (288). The VALIANT trial (276) and a case-control study reported at the ACC meeting in 2004 (279), attempted to clarify the relationship between ACEI and ARB in terms of use and risk of recurrence of MI and found no significant differences regarding the risk for recurring MI amongst the two treatment regimens. Three ongoing trials – ONTARGET (279), TRANSCEND (279) and ACCORD (289) – are anticipated to establish the role of ARBs in the treatment of high-risk patients for cardiac and vascular diseases.

11. Subcellular Remodeling due to MI

From the foregoing discussion, it is apparent that the MI results in cardiac hypertrophy with consequent CHF; however, the precise mechanisms by which these pathophysiological manifestations occur are not yet entirely comprehended. In regards to the changes in the phenotypic profile after an ischemic event, there are substantial alterations in immediate genes (119), Ca^{2+}-modulating proteins (112), contractile apparatus proteins (140), and proteins of the signal transduction communication pathways (290). The makeup of these disturbances are all linked with the process of remodeling the ventricular chambers in connection with altering cardiac contractile function, whereby diverse therapeutical compounds are conceived to transform the damaged tissue to enhance overall functioning of the heart (112,140,290). In view of the fact that it is not feasible to examine the entire registry of proteins it is very practical and hence crucial to investigate the chief target proteins involved in the process of excitation – contraction coupling in the myocardium in the failing state during the progression of CHF.

As part of the initiation of the remodeling process, the instantaneous instruction of encoding embryonic/fetal genes through transcription is pursued by hypertrophy of cardiomyocytes and eventual chronic HF in the rat model post ligation of the left anterior descending coronary artery. It is observed that there is an elevation in $[Ca^{2+}]_i$ during the diastolic phase and a reduction in the $[Ca^{2+}]_i$ during the systolic phase in the infarcted rat hearts. Nonetheless, it is not yet been established as to the changes in the SL Na^+-K^+ ATPase, SL Na^+-Ca^{2+} exchange, SR SERCA2a, SR PLB and SR RyR, in that they are not clearly clarified post MI. Features of neurohumoral stimulation, particularly of the SNS

Table 6. AT_1R Antagonists in Experimental Animals with MI

Animal Model	Drug	Dose	Therapeutic Effects	Reference
Male Wistar Rat CL	Losartan	1 mg/kg/day *icv*	Improved LV dimensions, EF, LVEDP, LVPSP and $dP/dt_{max/min}$ at 6 weeks.	Huang *et al.* [256]
		100 mg/kg/day *sc*	Only showed improvement of LVEDP at 6 weeks.	
		200 mg/kg/day *oral*	↓ AT_1R density in brain nuclei, improved LVEDP with an associated ↓ in LVPSP and dP/dt.	
Male S-D Rats CL	Losartan	20 mg/kg/day	Improved LVSP, $dP/dt_{max/min}$, systolic/diastolic aortic pressure. Also ↓ TIMP-1 protein levels, mitigated expression of ACE, AT_1R, attenuated myocardial fibrosis.	Xu *et al.* [257]
Male Wistar Rat CL	Irbesartan	50 mg/kg/day	Significantly ↓ LVEDP, LVESV, LVEDV, as well as ↓ interstitial fibrosis and expression of LV collagen I and III. Therapy combined with eplerenone ↑α/β MHC, ANF, SERCA2a levels.	Fraccarollo *et al.* [258]
Male S-D Rat CL	Losartan	20 mg/kg/day	Effectively ↓ HW, ascites, lung wet/dry wt ratio at 13wks. Attenuated LVSP, LVEDP, HR + Ca^{2+} uptake as well as Ca^{2+} stimulated ATP activity.	Shah *et al.* [259]
Male S-D Rat CL	Losartan	20 mg/kg/day	Corrected HW, HW/BW, lung wet/dry wt ratio as well as heart function – LVEDP, $dP/dt_{max/min}$. ↑ levels RyR, SERCA2a and PLB mRNA and protein.	Guo *et al.* [112]

Table 6 cont'd

Table 6 (Cont'd). **AT_1 R Antagonists in Experimental Animals with MI**

Animal Model	Drug	Dose	Therapeutic Effects	Reference
Male S-D Rat CL	Valsartan	15 mg/kg/day	Preserved LV FS%, systolic/diastolic diameters, and LVPW. Reversed changes in gene expression of muscle-specific, immune response, and fibrous tissue proliferation genes.	Gurevich *et al.* [260]
Female S-D Rat CL	Losartan	3 mg/kg/bid	Marked ↑ binding affinities of Ang II for AT_2R, with *NC* observed in AT_1R expression. Treatment also corrected HW/BW, LVW/BW, RVW/BW.	El-Sabban *et al.* [261]
Male Wistar Rat CL	Losartan	10 mg/kg/day	Significantly ↓ MAP, LVEDP. However, NC was observed in the mRNA levels of AT_1R and AT_2R levels at 30 min & 24 hrs post MI.	Zhu *et al.* [262]
Male Wistar Rat CL	Losartan	10 mg/kg/day	Attenuation of body characteristics of HW/BW in addition to improving LV dilatation & scar thinning.	Jain *et al.* [263]
Male S-D Rat CL	Losartan	2 g/L water	↓ LV and RV weights and improves MAP, LVSP. Successfully decreases interstitial fibrosis.	Thai *et al.* [264]
Male S-D Rat CL	Irbesartan	40 mg/kg/day	Reverses myocardial hypertrophy, attenuates ↑*tau* time constant of isovolumic relaxation, ANP levels.	Ambrose *et al.* [114]
Female S-D Rat CL	Losartan	2 g/L water	Effectively attenuates LV remodelling, inhibits elevation in TFG-β1 mRNA levels, ↓ interstitial fibrosis.	Youn *et a.l* [265]

Table 6 cont'd

Table 6 (Cont'd). **AT_1 R Antagonists in Experimental Animals with Myocardial Infarction**

Animal Model	Drug	Dose	Therapeutic Effects	Reference
Male Wistar Rat CL	Candesartan	1 mg/kg/day	Attenuates mRNA levels in LV and RV of ANP, α-skeletal actin, β-MHC, NCX, collagen I and III, and SERCA2a.	Hanatani *et al.* [81]
Male S-D Rat CL	Losartan	40 mg/kg/day	Limits collagen deposition, improves HW/BW, but shows no benefits in nonmyocytes, collagen deposition.	Taylor *et al.* [266]
Male S-D Rat CL	Losartan	2 g/L water	Similar effect as captopril in survival rate, yet linked with ↑ HR and ↓ peak developed pressure.	Milavetz *et al.* [267]
Male S-D Rat CL	Losartan	3 mg/kg/day	Treatment ↓ myocardial hypertrophy, preserves minimal coronary vascular resistance, reverses interstitial fibrosis in noninfarcted LV region.	Schieffer *et al.* [268]
Male Wistar Rat CL	Losartan	15 mg/kg/day	Significantly ↓ cardiac hypertrophy and fully inhibits deposition of collagen.	Smits *et al.* [269]
Male S-D Rat, CL	Losartan	40 mg/kg/day	Effectively ↑ venous compliance, ↓ LVEDP.	Raya *et al.* [270]

S-D – Sprague Dawley; **CL** – coronary ligation; **MI** – myocardial infarction; **icv** – intracerebroventricularly; **sc** – subcutaneously; **LV** – left ventricle; **LVPSP** – left ventricular peak systolic pressure; **HR** – heart rate; **RV** – right ventricle; **EF** – ejection fraction; **LVEDP** – left ventricular end diastolic pressure; **LVESV** – left ventricular end systolic volume; **LVEDV** – left ventricular end diastolic volume; **HW** – heart weight; **BW** – body weight; **Wt** – weight; RyR – ryanodine receptor; **SERCA2a** – sarco(endo)plasmic reticulum Ca^{2+} ATPase; **PLB** – phospholamban; **MHC** – myosin heavy chain; **AT_1R** – angiotensin II type 1 receptor; **NCX** – Na^{+}/Ca^{2+} Exchange; **NE** – norepinephrine; **ANP** – atrial natriuretic peptide; **NC** – no change observed.

Table 7. **Clinical Trials of AT_1R Antagonists in Patients with MI**

Clinical Trial	Drug Name	Human Dose	Study Highlights
ELITE [271]	Losartan	50 mg/day	Losartan have unexpected ↓ mortality than ACEI, and was better tolerated with fewer discontinued therapies.
RESOLVD [272]	Candesartan	4,8 or 16 mg/day	Alone was as efficient, tolerable and safe than ACEI, but in combination more beneficial to inhibit LV remodelling.
ELITE II [273]	Losartan	50 mg/day	Not chosen over BB for ↑HF survival in elderly, but better tolerated.
LIFE [274]	Losartan	50 mg/day	Prevents more CV deaths than BB with a similar ↓ blood pressure and is better tolerated.
OPTIMAAL [275]	Losartan	50 mg/day	No significant difference in mortality than ACEI in patients with acute MI and proof of HF with LV dysfunction.
VALIANT [276]	Valsartan	20 mg/twice daily 160 mg/twice daily	Alone is as effective as ACEI for ↓ CV risks after MI. Combination with ACEI ↑ side effects without ↑ survival.
CHARM-Alternative [255]	Candesartan	32 mg/day	Well tolerated drug which ↓ cardiovascular mortality and morbidity in patients with symptomatic chronic HF and intolerance ACE inhibitors.
CHARM-Preserved [277]	Candesartan	32 mg/day	Drug has moderate impact on inhibiting admissions for CHF with patients of HF and LVEF more than 40%.

Table 7 cont'd.

Table 7 (Cont'd). **Clinical Trials of AT_1R Antagonists in Patients with MI**

Clinical Trial	Drug Name	Human Dose	Study Highlights
CHARM-Added [278]	Candesartan	32 mg/day	Addition to ACE I gives clinically important ↓ in CV events in patients with CHF and ↓ LVEF.
ONTARGET [279]	Telmisartan	80 mg/day	Ongoing trial to see if ARB + Ramipril more effective than ramipril alone; if Telmisartan as effective as ACEI alone.
TRANSCEND [279]	Telmisartan	80 mg/day	Ongoing trial to see if ARB more effective than placebo in patients who cannot tolerate ACEI.

ELITE – Evaluation of Losartan in the Elderly Study; **RESOLVD** - The Randomized Evaluation of Strategies for Left Ventricular Dysfunction; **LIFE** – Losartan Intervention for Endpoint Reduction; **OPTIMAAL** – The Optimal Therapy in Myocardial Infaction with the Angiotensin II Antagonist Losartan; **VALIANT** – Valsartan in Acute Myocardial Infarction; **CHARM** – Candesartan in Heart Failure, Assessment of Reduction in Mortality and Morbidity; **ONTARGET** – Ongoing Telmisartan Alone and in Combination with Ramipril Global Endpoint Trial; **TRANSCEND** – The Telmisartan Randomized Assessment Study in Ace Intolerant Subjects with Cardiovascular Disease; **ACEI** – Angiotensin Converting Enzyme Inhibitor; **BB** – β-blocker; **ARB** - Angiotensin Receptor Blocker; **HF** – heart failure; **CHF** – congestive heart failure; **MI** – myocardial infarction ; **LV** – left ventricular; **CV** – cardiovascular; **LVEF** – left ventricular ejection fraction.

and RAS, are imperative in issues of ventricular modifications during the advancement of myocardial thickening and hypertrophy towards CHF. The activation of these compensatory systems not only influences the topographic remodeling of the ventricles, but also impinges on the cardiomyocyte workload in the disease state associated with MI. Through an assortment of clinical trials in tandem with animal model experimentation, inhibition of the SNS and RAS with α-antagonists, β-antagonists and AT_1R antagonists respectively, has effectively been proven to give advantageous results in CHF due to MI. The valuable measures of SNS and RAS blockade on cardiac contractile performance in the failing heart are believed to be a direct result in the overall capability to control fibrinous scar and infarct parameters, decrease the expansive capacity of the infarct to spread, hence reducing the amount of ventricular shear stress placed on the myocardial wall, in addition to inhibiting and potentially reversing cardiac subcellular remodeling post MI. Various investigations have also revealed that both the above mentioned groups of pharmacological therapies have similar effectiveness and acceptability amongst subjects experiencing the moderate and severe stages of CHF. In light of the phenomenon that changes intracellularly involving subcellular organelles of the SR, myofibrillar components and SL in CHF due to MI are virtually attenuated by α-/β- antagonists and AT_1R antagonists, it is therefore proposed that the valuable effects of SNS and RAS blockade in the failing heart may be possibly a direct result of these particular drug targets and their corresponding actions on the remodeling of subcellular entities in the myocardium.

II. STATEMENT OF THE PROBLEM AND HYPOTHESIS TO BE TESTED

During the pathological course of the infarcted heart, it has been acknowledged that MI leads to cardiac subcellular remodeling, which is associated with changes in the genome expression for the occurrence of molecular, cellular and interstitial alterations that are intrinsic to the size, shape and function of the myocardium. After the initial insult of an MI, it is widely accepted that activation of the RAS and SNS follows, which in turn play a key role in the cardiomyocyte subcellular and molecular modifications. Consequently, it is hypothesized that blockade of the SNS and RAS may not only cease the damage done to the myocardium, but in fact reverse the process of cardiac subcellular remodeling and hence promote improved cardiac contractile function.

Although the heart functions as a whole with its four chambers, the insult of an MI promotes the rearrangement of the architecture of the most important chamber, the LV. The specific measurements of the IVS, LVID and LVPW throughout diastole and systole are critical to the well being of this organ. However, during the course of CHF the ventricular chamber begins to dilate, the LV wall becomes hypertrophied and portions of the outer wall become thinned out. As most of this knowledge has been documented in past reports, there has been little mention of the internal cardiac structure of the infarcted rat heart 12 wks post MI. The aim of this study is to institute drug treatment once CHF is firmly established, in order to test whether these agents can significantly reverse cardiac subcellular remodeling in CHF due to MI. Due to the fact that pharmacological intervention limits infarct size, it is our goal to allow for full development of the

infarction, along with its associated reorganization, before treatment is implemented. This not only makes our study unique in its design, but it also mimics the real life clinical scenario of a patient who has already experienced myocardial damage from an MI, and focuses on his point of view as the MI progresses toward CHF. Furthermore, we propose to characterize the structural design of the heart at this time period of 12 wks to be able to determine whether these changes can be attenuated with the therapeutic treatment for 8 wks with agents designed to block the SNS and RAS. In addition, we also wish to determine overall cardiac performance, in association with hemodynamical studies at baseline levels, and to compare that with treatment groups of losartan, metoprolol and prazosin, in order to prove partial attenuation of these deficits with blockade of the SNS and RAS. The chief components of the SNS and RAS that carry out the hazardous effects of the disease process include norepinephrine, epinephrine, and dopamine as well as Ang II (231). In order to improve cardiac energy metabolism and avoid further overstimulation of these two systems, blockade of the appropriate downstream receptors is necessary. Thus, the present study was undertaken to appraise the beneficial effects on myocardial bioenergetics by testing the hypothesis of β-, α- and Ang II-blockade in reversing changes in myocardial structure and function in CHF due to MI.

It is well established that cardiac subcellular remodeling has been linked with changes in the SR SERCA2a and PLB proteins (291). In the case of SERCA2a in the infarcted rat hearts, cardiac contractile dysfunction begins with alterations at the level of gene expression (77,108-112,120), where there exists a clear depression in the amount of mRNA expressed. This can be further associated with a decrease in protein expression (77,108,109,112,116,120,292), giving rise to a larger PLB/SERCA2a protein ratio

allowing a greater negative regulatory effect of PLB on SERCA2a. As the interaction between these two neighboring SR proteins ultimately controls the $[Ca^{2+}]_i$ content and contractility of the cardiomyocyte, this ratio must remain in a harmonized equilibrium (293). As previous studies have reported irregularities in the balance of PLB/SERCA2a through altered levels of gene expression, protein expression, and functional capacity, the mechanism of reduced SERCA2a activity still remains to be elucidated. SERCA2a has the primary responsibility of maintaining Ca^{2+} homeostasis in and out of the cytosol of the cardiomyocytes (46, 294), and is composed of three genes: SERCA1 (fast-twitch skeletal muscle), SERCA2 (predominantly myocardium), SERCA3 (specialized tissue) (294). Much information is available regarding these various gene types in different models of CHF, yet the literature is scarce with respect to the status of the SR SERCA2a and PLB mRNA, protein, and activity levels, in CHF due to MI. Hence, it is proposed to evaluate the various modifications in the SR Ca^{2+} uptake activity and link them directly with changes in the protein and genetic molecular levels to reveal the core development of CHF due to MI. Further testing will disclose if these modifications can be attenuated with the treatment of either losartan, metoprolol or prazosin.

The SNS and RAS systems are key players involved in both animal model experiments and clinical studies, with their blockade becoming an attractive therapeutic technique in CHF due to MI. Nonetheless, the mechanism underlying the effects of the SNS and RAS on SR Ca^{2+} functioning in the MI state has not been completely elucidated. In order to better understand the beneficial outcomes of SNS and RAS antagonism on the basis of SR Ca^{2+} transport, we will determine if the alterations in cardiac function of CHF-induced LV remodeling are linked to irregular SR SERCA2a function, and if these

changes can indeed be reversed with pharmacological intervention with losartan, metoprolol or prazosin. In response to pathological stimuli, the α- and β-isoform cardiac MHC genes have been reported to transform their occurrence in pattern expression severely (295). As a direct consequence to the threat of tissue death, a transitional isoform shift takes place in the MHC composition from α- to β-predominance at the point of transcriptional production of mRNA (108,140). Though this phenomenon of myofibrillar remodeling transpires alongside other organelle remodeling, the means by which this conversion unfolds requires further investigation. Subsequently, a portion of this study will be devoted to assessing whether the damage caused by the activation of the SNS and RAS can be partially reversed using the compounds of losartan, metoprolol or prazosin to see is there is an isoformal shift in cardiac MHC back toward the original baseline values. By addressing these objectives, this study aims to provide a comprehensive representation of how SNS and RAS blockade can reverse cardiac subcellular remodeling at the genetic, protein, cellular and functional levels during the clinical course of CHF due to MI in the Sprague-Dawley rat animal model.

III. MATERIALS AND METHODS

1. Experimental Model

All experimental protocols employed in this study have been approved by the Animal Care Committee of the University of Manitoba following the ethical guidelines established by the Canadian Council on Animal Care. The experiments were performed at the St. Boniface General Hospital Research Centre, in agreement with the "Guide to the Care and Use of Experimental Animals". CHF due to MI was induced in Sprague-Dawley rats (175 - 200g) by occlusion of the left anterior descending coronary artery as previously used in our laboratory (112,115,296-298).The rats were anesthetized with 2.5 % isoflurane gas on 2L oxygen along with intermittent positive pressure ventilation. The fourth and fifth ribs were incised adjacent and to the left of the sternum. The pericardial sac was removed from its location around the heart and the heart was gently exposed from the thoracic cavity. Using a 6-0 silk suture, the left anterior descending coronary artery was ligated approximately 2 mm from the origin of the aorta, and the heart was then repositioned back into the chest. In order to remove the remaining air in the thoracic cavity to initiate proper respiration for recovery, a syringe was inserted into the thorax, as the chest muscles were securely closed with a purse-string. The rats then received a mixture of 95% O_2 and 5% CO_2 that was administered under positive pressure. The sham rats were treated in the same manner, except that the coronary suture was not tied.

The incidence of mortality of these rats was approximately 30-36% within the initial 48 hours. The animals were assessed electrocardiographically both before and after the surgery to determine the extent of coronary artery ligation whereas echocardiography was employed at 12 wks post surgery before the commencement of drug intervention,

and again at the end time point of 20 wks post surgery.

2. Treatment with Losartan, Metoprolol or Prazosin

After completion of surgeries, all rats received the same standard care of 12 hr day/night cycle and had access to food and water *ad libitum*. The animals were then randomly divided into 8 groups for this particular project: sham control, infarcted (MI), sham treated with losartan (Sham + LOS), infarcted treated with losartan (MI + LOS), sham treated with metoprolol (Sham + MET), infarcted treated with metoprolol (MI + MET), sham treated with prazosin (Sham + PRAZ), and infarcted treated with prazosin (MI + PRAZ). Twelve wks after the surgery, losartan (20 mg/kg/day), metoprolol (50 mg/kg/day) or prazosin (10 mg/kg/day) were administered orally for a period of 8 wks. Sham control animals received tap water without any drug. Losartan, metoprolol and prazosin were dissolved in the appropriate amount of tap water; all solutions were made fresh daily.

3. Echocardiographic Assessment of Cardiac Performance

Cardiac ultrasound studies were performed using the SONOS 5500 ultrasonograph (Agilent Technologies). On the day of the study, rats were anaesthetized with 2.5 % isoflurane gas on 2L oxygen, and were allowed to breathe spontaneously. The chest area was shaved with electric clippers and subsequent echocardiographic readings were recorded with animals lying on their left side. A 12-MHz annular array ultrasound transducer was gently positioned on the coupling gel over the hemithorax to allow appropriate contact, without applying excessive pressure on the thoracic region.

Transthoracic short axis measurements were performed in the left lateral decubitus position, while the transducer was gently rotated to achieve the optimal position. In the parasternal short axis orientation, the probe recorded left ventricular diastolic and systolic measurements. The M-mode echocardiograms at the papillary muscle level measured the following parameters: interventricular septum diastole/systole thickness (IVSd, IVSs), left ventricular internal diameter diastole/systole (LVIDd, LVIDs), left ventricular posterior wall diastole/systole thickness (LVPWd, LVPWd), ejection fraction (EF), fractional shortening (FS), cardiac output (CO), and heart rate (HR).

4. Hemodynamic Studies *in vivo*

The procedure involving hemodynamical measurements is a terminal study, and was therefore carried out on the day of sacrifice. Sham, MI and drug-treated rats were assessed for left ventricular function and overall cardiac performance. Rats were anaesthetized using an intraperitoneal injection containing a cocktail of ketamine:xylazine (100:10 mg/kg). The right carotid artery was exposed and a micromanometer-tipped catheter (2-0: Millar SPR-249) was gently inserted and advanced into the left ventricle (LV). A silk ligature was used to secure the catheter to give proper recordings. After a period of cardiac stabilization, systolic pressure, diastolic pressure, mean arterial pressure (MAP), as well as maximum rates of isovolumic pressure development (+dP/d*t*) and pressure decay (-dP/d*t*) were calculated and recorded (112,115,149,290,296-298). Hemodynamic data was to be measured and recorded instantaneously using the AcqKnowledge program for Windows 3.03 (MP100, BIOPAC Systems, Inc., Goleta, CA, USA). After the hemodynamic procedure was completed, the

hearts were removed from the chest cavity, and the LV with the septum as well as the scar tissue were dissected, weighed and immediately frozen in liquid nitrogen and stored at -80^0 C until further use.

5. Tissue Preparation and Overall Body Assessment

Upon completion of the hemodynamical studies, the abdominal cavity was opened in order to remove the liver and kidneys, in addition to the removing the lungs from the thoracic cavity. All organs were washed twice in a solution of 0.9% NaCl, weighed and placed on drying dishes. The hearts were quickly excised from the chest and washed twice in a solution of 10 mM 3-[N-morpholino]-propanesulfonic acid (MOPS) and 10 mM sodium ethylenediaminetetraacetate (EDTA). The LV along with the septum, the RV, and the scar tissues were carefully dissected from the whole heart, washed again in solution, weighed, frozen in liquid nitrogen and stored at -80^{0}C for future use. This whole operation was performed in ice buckets with ice in a period of 2 min to ensure protection of tissue during it handling. The lung weights were measured at the time of excision and again when they were dry to give a wet /dry ratio which illustrated an index of pulmonary congestion in various rat groups. Additionally, the ratio of heart weight (both ventricles and infarct scar tissue) to total body weight provided an index of cardiac hypertrophy in all animal groups. The scar tissue that developed in the LV was carefully removed and stored in a separate container from the still viable LV. The size of the infarct was established, and the remaining functional LV and its septum were kept for all biochemical studies. The ratio of scar weight/total LV weight illustrated a fairly linear association with the size of the infarct, as was determined morphometrically (112,299);

the scar weight was to be used as a marker to quantify the extent of the scar tissue size (112,115,296-298).

6. RNA Isolation

Using the TRIzol ® Reagent method (GIBCO BRL, Burlington, ON, Canada), total RNA was isolated from LV tissue from all animal groups. The frozen LV tissue was taken from -80^{0}C and was placed on ice at -20^{0}C until the start of the procedure. Individually, each sample was removed from storage, chopped with scissors and weighed to give a final sample of 150-200 mg. In a mortar, the tissue was quickly crushed into a powder by a pestle as liquid nitrogen was continuously added. After the addition of 2 μl of TRIzol reagent, this mixture was aliquoted into 50 ml Corningware tubes and further homogenized. Homogenization by the PowerGen 125 (Fisher Scientific) lasted for 20 sec with a 10 sec rest in between, and was essentially repeated 5 times. Samples from all groups underwent this same process, and the residual tissue was further returned to storage of -80^{0}C. Subsequently, the homogenate was equally poured into two centrifuge tubes. Upon the addition of 200 μl of chloroform to each tube, the mixtures were inverted by hand 50 times and then centrifuged at 4^{0}C at 10, 000 g for 20 min. The supernatant was then discarded and the pellet was further re-suspended with 1.0 ml of 75% ethanol, followed by gentle agitation with a pipette. Further centrifugation at 4^{0}C at 10,000 g was carried out for a period of 10 min. After the supernatant was carefully poured off, the inside of the tube was gently wiped with a Kimwipe. The consequent pellets were allowed to dry at room temperature for a total of 20 min, followed by reconstitution with 50 μl nuclease free water. Using a pipette tip, the mixture was further agitated 50 times

and the remaining solution was then heated in an Isotemp 500 Series Laboratory Oven (Fisher Scientific) at 55^0C for 10 min. The samples were then all placed on ice; 4.4 µl was added to an Eppendorf tube of 996 µl RNAse free water. Using the Spectronic 601 Spectrophotometer (Fisher Scientific), the RNA concentrations was quantified first at 260 nm, and then again at 280 nm. Accordingly, the RNA concentration was calculated as 260 nm/280 nm ratio. A concentration value of 1.6 µg/µl or greater was therefore considered adequate. Following this, the RNA samples were stored at -80^0 C until further use.

7. Northern Blot Analysis and Molecular Probes

Total RNA was extracted from heart tissue using the TRIzol ® Reagent. Samples of total RNA in sterile distilled water with 0.2% DEPC were denatured at 65^0C for 10 min in 50% formamide, 7% formaldehyde, 20 mM MOPS (pH 7.4), 2 mM EDTA (pH 8.0) and 0.1% SDS. This was then followed by electrophoresis in a 1.2% agarose/formaldehyde gel to size fractionate the mRNA transcripts. In this study, 20 µg of total RNA were used. Gel electrophoresis was immediately followed by transfer of the fractionated mRNA transcripts to a positively charge-modified nylon filter (NYTRAN ® PLUS, Schleider and Schuell, Keene, NH, USA) via capillary action for a period of 24 hr. The nylon membrane was then promptly UV covalently cross-linked (UV Stratalinker 2400 Stratagene). The blots were prehybridized to random primed cDNA or oligonucleotide probes in a solution of 50% formamide, 10% Denhardt's solution, 1% SDS, 0.2 mg/ml denatured salmon sperm DNA, 10 mM EDTA (pH 8.0), 25% '4 x RNA' solution [3 M NaCl, 0.6 M Tris-HCl (pH 7.5), 0.18 M NaH_2PO_4, 0.24 M Na_2PO_4, 0.1 M

$Na_4P_2O_7$] at 42^0C for 6-16 hr. Hybridization was performed overnight in the INNOVA 4000 incubator (New Brunswick Scientific, Edison, NJ, USA) with an oscillation rate of 65 rpm in the presence of ^{32}P-labelled specific probes. SERCA2a was probed with a 0.762 kb cDNA fragment from the rabbit heart Ca^{2+}-pump ATPase (courtesy of Dr. A.K. Grover, McMaster University, Hamilton, Canada). PLB was probed with a 0.153 kb cDNA fragment from the rabbit heart (courtesy of Dr. D.H. MacLennan, University of Toronto, Toronto, Canada). CQS was probed with a 2.5 kb cDNA fragment from the rabbit heart (courtesy of Dr. A. Zilverman, University of Cincinnati, Cincinnati, OH, USA). For myocardial MF α- MHC, a 39-meroligonucleotide derived from the 3`-untranslated region of the rat α-MHC gene had the following sequence: 5`-GGG ATA GCA ACA GCG AGG CTC TTT CTG CTG GAC AGG TTA-3`(American Type Culture Collection, Rockville, MD USA), and for the myocardial MF β-MHC probe, a 30-meroligonucleotide was derived from the 3`-untranslated region of the gene (specifically rat genome) and was the following sequence: 5`CAG GCA TCC TTA GGG TTG GGT AGC ACA AGA-3` (American Type Culture Collection, Rockville, MD, USA). In addition, synthetic oligonucleotides were 5' end radio-labeled with T4 polynucleotide kinase (GIBCO-BRL Life Technologies, Burlington, Ontario, Canada) and γ-^{32}P [ATP] (DuPont NEN™ Life sciences Products, Boston, MA, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a 1.2 kb cDNA fragment of the human GAPDH (American Type Culture Collection, Rockville, MD, USA), as well as 18S, a 24 base oligonucleotide probe (5'ACGGTATCTGATCGTCTTCGAACC –3') of rat 18S ribosomal RNA, were used as an internal standard to account for the differences in nucleic acid loading and transfer of the RNA. Washing of the membranes with 1X

standard saline citrate and 0.1% SDS was carried out at room temperature at an oscillation rate of 64 rpm. The membranes were then exposed to Kodak X-Omat-AR film using intensifying screens at –70° C. The radio-labeled mRNA bands were then scanned using a densitometer GS-800 (BIO-RAD, California, USA) and were quantified with the Quantity One 4.4.0 software (BIO-RAD, USA).

8. Preparation of Cardiac SR Fraction

SR vesicles from the cardiac muscle were isolated as previously described (300,301). Once the tissue samples were removed from the -80^0 C storage, they were each weighed and placed in a centrifuge tube that was placed on ice. To each sample was added 10 ml/g of a buffer solution containing (in mM): 10 $NaHCO_3$, 5 NaN_3, 15 Tris-HCl (pH 6.8) and protease inhibitors (in μM): 1 leupeptin, 1 pepstatin and 100 phenylmethul-sulfonylflouride. The tissue was further minced using scissors, and then immediately homogenized twice for a period of 20 sec with a Polytron homogenizer (Brinkman, Westbury, NY) that was set at half the maximal setting. Shortly after the homogenization process, the homogenate was promptly centrifuged at 4^{0}C for 20 min at 9,500 rpm (Beckman, JA 20.0) to remove all cellular debris. The supernatant was then centrifuged at 4^{0}C for 45 min at 19,000 rpm (Beckman, JA 20.0). Subsequently, this supernatant that contained the cytosolic fraction was further aliquoted into Eppendorf tubes and stored at – 80^0 C. The remaining pellet was suspended in a buffer containing 0.6 M KCl, and 20 mM Tris-HCl pH 6.8, and centrifuged at 4^{0}C for 45 min at 19,000 rpm (Beckman, JA 20.0). The final pellet that contained the SR fraction was suspended in 250 mM sucrose and 10 mM histidine pH 7.0, aliquoted into Eppendorf tubes and stored at -80^{0}C. It is

important to note that the entire procedure of the isolation of the SR vesicles was carried out at 4^0C.

9. Protein Content Determination

Determination of the protein concentration of the SR vesicles was completed using Lowry's method. Various concentrations of bovine serum albumin (BSA) in deionised distilled water were used as standards to generate a standard curve. 2 ml of working solution that contained 2% potassium sodium tartrate, 1% $CuSO_4$ and 2% Na_2CO_3 (in 0.1 N NaOH) in a ratio of 1:1:100 were added to the standards, blank and samples followed by immediate vortexing. After a period of 10 min, 0.2 ml of 1N phenol reagent (Folin and Ciocalteau reagent) was added in each tube and 20 min later, the absorbance of each tube at 623 nm was measured using the Ultrospec 2100 pro spectrophotometer (Biochrom). The protein concentration was therefore determined by the standard curve of BSA using a custom-made computer software program in Microsoft Excel.

10. Measurement of SR Ca^{2+}-uptake and Ca^{2+}-release activities

The SR Ca^{2+}-uptake activity was measured using a previously described procedure (302). For a total volume of 250 µl, the reaction mixture containing (in mM): 50 Tris-maleate (pH 6.8), 5 NaN_3, 5 ATP, 5 $MgCl_2$, 120 KCl, 5 K-oxalate, 0.1 EGTA, 0.1 $^{45}CaCl_2$ (12,000 cpm/nmol) and 0.25 ruthenium red was prepared. The Ca^{2+}-uptake reaction mixture was initiated upon the addition of SR membranes (20 µg) from each treatment group, and was incubated at 37^0C for 2 min. Termination occurred at the third

min, as the reaction mixture plus the SR sample was then filtered in 200 µl aliquots through 0.45 µm Millipore filters. Immediately following filtration, the individual filters were washed twice with 3 ml of a washing buffer and then promptly dried for 1 hr at 60^0C. The filters were then be placed into labelled scintillation vials, where 10 ml of scintillation fluid was added to each vial before they were counted in a beta liquid scintillation counter.

The determination of the Ca^{2+}-release activity of the isolated SR vesicles was carried out by a modified procedure as descrived previously (303,304). SR vesicles were initially suspended in a reaction medium with total volume of 625 µl that consisted of (in mM): 62.5 µl of 0.5mg/ml SR, 100 KCl, 5 $MgCl_2$, 5 potassium oxalate, 5 NaN_3 and 20 Tris-HCl (pH 6.8). Next, the SR fraction was incubated for a period of 45 min at room temperature in a mixture of 10 µM $^{45}CaCl_2$ (20 mCi/L) and 5 mM ATP. Subsequently, Ca^{2+}-induced-Ca^{2+}-release activity was performed via the addition of 1 mM EGTA plus 1 mM $CaCl_2$. The ongoing reaction was promptly terminated 15 sec later using the Millipore filtration technique. The filters were placed in labeled vials that contained 10 ml of scintillation fluid. The vials were then immediately placed in a beta-scintillation counter and counted accordingly. The Ca^{2+}-induced Ca^{2+} release was completely prevented (95% to 97%) by the treatment of 20 µM ryanodine to the SR preparations.

11. Western Blot Analysis

The relative protein contents of the SR Ca^{2+}-ATPase (SERCA2a), phospholamban (PLB), and calsequestrin (CQS) were measured as previously described (301). Protein samples were suspended in a 1:1 ratio with Laemmli buffer that contained: 0.1 M Tris-

HCl (pH 6.8), 15% (w/v) sodium dodecyl sulphate (SDS), 15% glycerol, 8% β-mercaptoethanol and 0.002% bromophenol blue. The solution was subsequently denatured in boiling in water for 3 min. SR protein samples (1 mg/ml) were separated by SDS-PAGE on a 15% gel for PLB, 12% gel for CQS, and 10% gel for SERCA2a. Each group contained sample loads of the same volume (10 μl in each well). The protein bands were further transferred to polyvinylidene diflouride membranes (Millipore Corporation, Bedford, MA, USA) at 200 V for 1-2 hr. The transfer buffer included 25 mM Tris-HCl, 192 mM glycerine and 4% methanol (v/v). After the transfer procedure, the membranes were incubated overnight at 4°C in blocking buffer (TBS: 10 mM Tris and 150 mM NaCl, combined with 5% fat-free powered milk). The next day the membranes were subsequently incubated at room temperature for 1 hr in monoclonal anti-SERCA2a (Affinity Bioreagents, Inc., Golden, CO, USA), monoclonal anti-phospholamban (Upstate Biotechnology, Lake Placid NY, USA), monoclonal anti-calsequestrin (Upstate Biotechnology, Lake Placid, NY, USA). The membranes were then incubated at room temperature for 45 min with a secondary antibody. For SERCA2a, PLB, and CQS a biotinylated anti-mouse IgG antibody (Amersham Life Science, Oakville, Ontario, Canada) was the agent of choice. Finally, the membranes were all incubated for 30 min at room temperature with a streptavidin-conjugated horseradish peroxidase (Amersham Life Science, Oakville, ON, Canada) in Tris-buffered saline with 0.1% Tween-20 (TBST). In between the incubation with antibodies, the membranes were washed a total of 3 times with TBST for a period of 15 min. The antigen-antibody complex incorporated in each membrane was detected using the chemiluminesence ECL kit (Amersham Corporation, Arlington Heights, IL, USA). The protein bands were then visualized on Hyperfilm-ECL

(Amersham Corporation, Arlington Heights, IL, USA). These values were promptly scanned using a densitometer GS-800 (BIO-RAD, Hercles, California, USA) and were further quantified with the Quantity One 4.4.0 software (BIO-RAD, USA).

12. Plasma Catecholamine Determination by HPLC

The plasma catecholamine levels of norepinephrine, epinephrine, and dopamine were measured using high-performance liquid performance chromatography (HPLC) as previously described (305). Catecholamines in the plasma portion of each sample were extracted by using activated aluminum oxide and measured by employing HPLC with electrochemical detection. This particular chromatographic apparatus incorporated a Spectra-Physics SP 8700 solvent delivery system with an LC-4A amperometric detector in the mode for oxidation (Bioanalytical Systems). The column was described as a NOVA PAC^{TM} C_{18} (with corresponding part number 086344) reverse phase (Water Associates). The consequent results were tabulated using a Hewlett-Packard integrator 3390A. This technique of HPLC was coupled with electrochemical detection that offered high sensitivity and specificity to the level of pictogram, for the detection of plasma catecholamine levels.

13. Myofibrillar Ca^{2+}-stimulated and Mg^{2+}-ATPase Activity

The isolation of the MF fraction was carried out as previously described (139). Briefly, the isolated MF fraction was suspended in a final solution of 100 mM KCl and 20mM Tris-HCl (pH 7.0).The Mg^{2+}-ATPase activity was measured at 30 ^{0}C in a medium that consisted of (in mM) 20 imidazole (pH 7.0), 2 Mg_2Cl_2, 2 Na_2ATP, 10 NaN_3, 1.6

EGTA and 50 KCl (139). In the same medium, the total ATPase activity was evaluated, except that the EGTA was essentially replaced with 10 μM of free Ca^{2+}. The Ca^{2+}-stimulated ATPase activity was determined as the difference between the values obtained for the total and Mg^{2+}-ATPase activities. All ongoing reactions were terminated at the 5 min period by the incorporation of 12% tricholoroacetic acid. The subsequent samples were centrifuged at 1000 g, at which time the amount of phosphate in the supernatant was determined using the colorimetric method.

14. Statistical Analysis

All the results were expressed as mean ± SE. Differences between the sham, MI- and drug-treated animal groups were evaluated by the Analysis of Variance (ANOVA) test, followed by the Newman-Keuls test. Statistical analysis was performed with Microcal Origin version 6 (Microcal Software, Northampton. MA), and a probability level of $p < 0.05$ was considered the threshold for statistical significance.

IV. RESULTS

1. Echocardiographic assessment of cardiac function 12 wks post MI

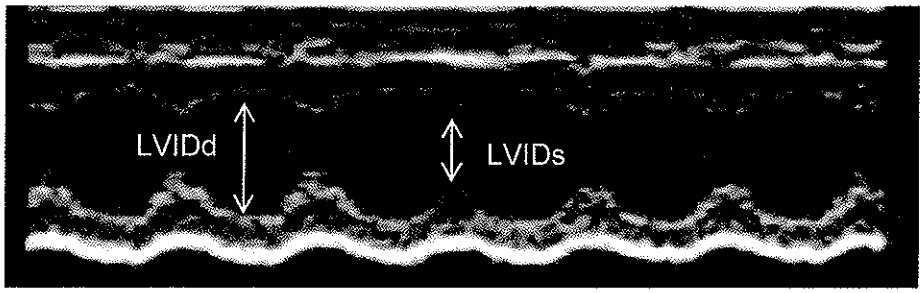
The echocardiographic *in vivo* parameters measured at 12 wks post coronary artery ligation showed significant reductions in EF, FS, and CO (Figure 4; Figure 5, panels A-D), indicating a marked depression in cardiac performance. The internal dimensions of the cardiac chamber were also measured showing noteworthy alterations in LVIDd, LVIDs, IVSs, LVPWd and LVPWs without any changes in IVSd (Table 8). These changes in LV dimensions indicated cardiac remodeling in 12 wks post MI animals. Thus, 12 wks post MI rats were treated with or without various agents for a period of 8 wks to test if these treatments were effective in reversing different alterations associated with HF.

2. Effects of AT_1R Antagonist on Remodeling in the Failing Rat Heart

a. Attenuation of General characteristics due to MI

The occlusion of the left anterior descending coronary artery for 20 wks produced a scar in the left ventricular chamber, while the surrounding cardiac muscle underwent hypertrophy as reflected by enlarged values for the heart wt compared to control (Table 9). The heart wt, which consisted of the LV, RV and scar, in addition to the heart wt/body wt ratio were significantly increased due to the development of cardiac hypertrophy 20 wks post MI. The cardiac hypertropy parameters were significantly attenuated by losartan treatment (Table 9). The mean scar weight varied from 700 mg to 800 mg with no major difference amongst the various groups of the MI rats, which equates to a mean scar size value of approximately 44%, as reflected by the scar

A) Control at 12 weeks



B) Myocardial infarction at 12 weeks

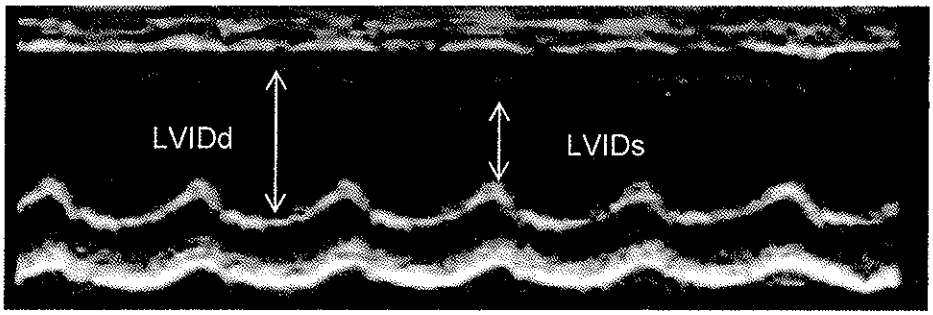


Figure 4. M-Mode echocardiographic images of internal cardiac dimensions of A) Sham rats, and B) Myocardial infarcted rats at the time point of 12 weeks post coronary artery occlusion; LVIDd – left ventricular internal diameter – diastole; LVIDs – left ventricular internal diameter – systole.

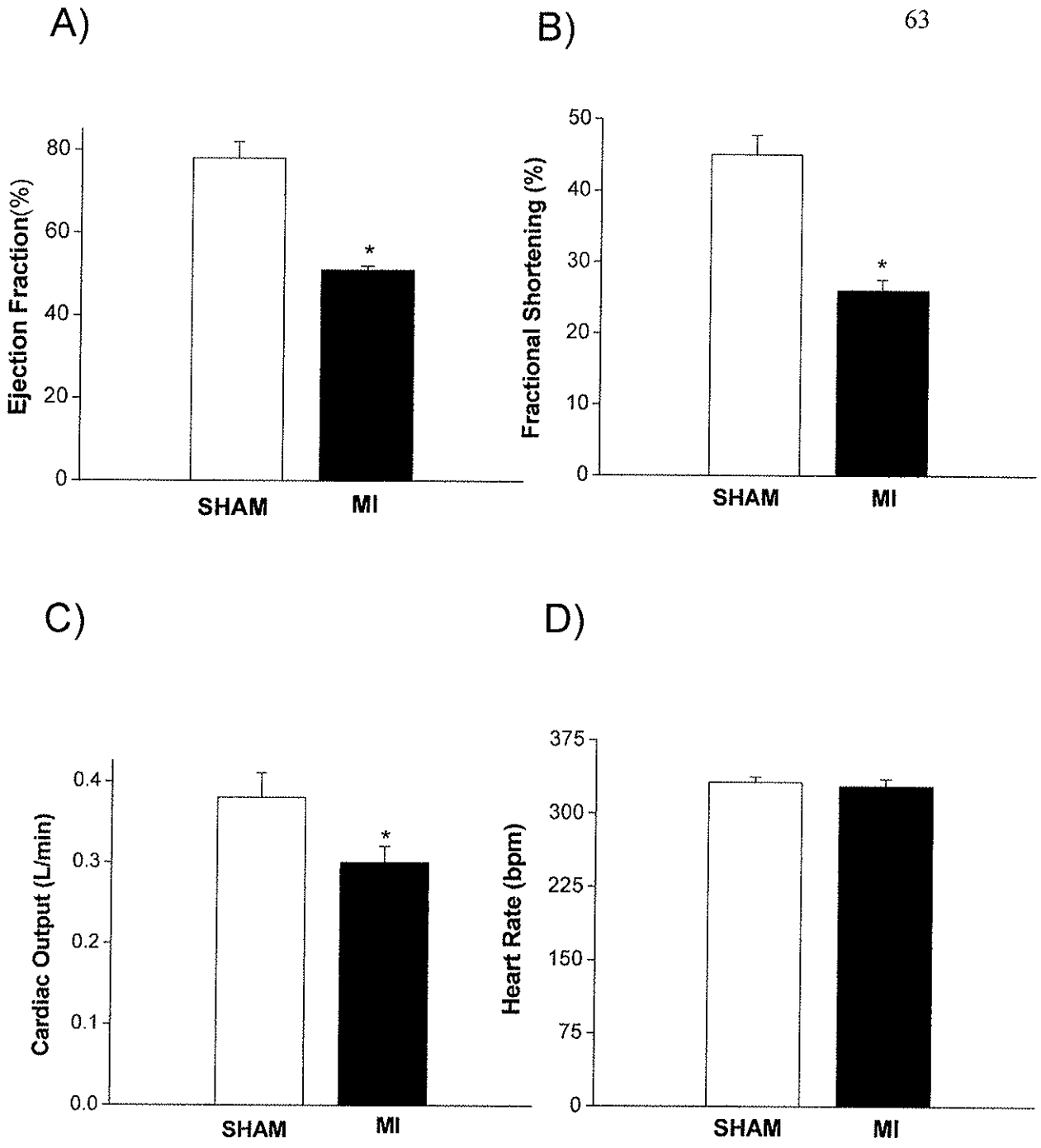


Figure 5. Assessment of cardiac function by echocardiography of sham and MI rats 12 weeks post coronary artery occlusion. A) Ejection fraction. B) Fractional shortening. C) Cardiac output. D) Heart rate. *p < 0.05 compared with sham (n = 20).

Table 8. **Echocardiographic assessment of internal cardiac dimensions during systole and diastole in sham and MI rats 12 weeks after coronary artery occlusion.**

Parameter	Sham	MI
IVSd (cm)	0.20 ± 0.01	0.22 ± 0.02
IVSs (cm)	0.36 ± 0.02	0.28 ± 0.01*
LVIDd (cm)	0.83 ± 0.03	1.01 ± 0.01*
LVIDs (cm)	0.46 ± 0.03	0.76 ± 0.01*
LVPWd (cm)	0.24 ± 0.02	0.35 ± 0.02*
LVPWs (cm)	0.32 ± 0.02	0.39 ± 0.01*

Values are a mean ± SE of 20 animals in each group. MI – myocardial infarction; IVSd – interventricular septum, diastole; IVSs – interventricular septum, systole; LVIDd – left ventricular internal diameter, diastole; LVIDs – left ventricular internal diameter, systole; LVPWd – left ventricular posterior wall, diastole; LVPWs – left ventricular posterior wall, systole. *$p < 0.05$ compared with sham.

Table 9. **General characteristics of sham and infarcted rats with and without losartan treatment for 8 weeks starting at 12 weeks after coronary artery occlusion.**

Parameter	Sham	MI	Sham + LOS	MI + LOS
Body wt (g)	680 ± 29	684 ± 30	709 ± 45	692 ± 16
Heart wt (mg)	1616 ± 64	2600 ± 86*	2166 ± 37*	2129 ± 64#
Scar wt (mg)	ND	750 ± 130	ND	516 ± 87
Scar wt/LV wt (%)	ND	44 ± 6	ND	38 ± 6
Heart wt/body wt (mg/g)	2.85 ± 0.11	4.18 ± 0.19*	3.11 ± 0.50	3.26 ± 0.07#
RV wt (mg)	375 ± 20	635 ± 60*	283 ± 31	371 ± 18#
Lung wet/dry wt ratio	4.08 ± 0.18	5.13 ± 0.24*	4.15 ± 0.10	3.56 ± 0.28#
Liver wet/dry wt ratio	2.68 ± 0.07	2.90 ± 0.03	3.07 ± 0.16	2.82 ± 0.05

Values are mean ± SE of 7 animals in each group. MI – myocardial infarction; LOS – Losartan (20 mg/kg/day); ND – not detected; wt – weight; 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.

wt/LV wt ratio. The cardiac architecture also showed key changes in chamber dimensions, where the RV weight showed marked thickening (increase of 69%), which was greatly diminished with losartan to suggest a reduction in the degree of overall cardiac remodeling. Table 9 showed no significant changes in total body weight, scar weight and scar weight/LV weight ratio amongst various infarcted and drug-treated groups when compared to sham rats. Although heart wt of sham animals was significantly increased upon treatment with losartan, the heart wt/body wt ratio was not altered (Table 9). The data in Table 9 also showed an alteration in the lung wet/dry wt ratio, with an increase of 25% of the sham rats to suggest a considerable amount of pulmonary congestion and edema in MI animals. Treatment with losartan demonstrated substantial recovery of the lungs during cardiac failure. However, no changes were observed for the liver wet/dry weight ratio due to MI in drug-treated or untreated groups (Table 9).

b. Cardiac performance, subcellular activities and ventricular remodeling

The 20 wks infarcted hearts showed a depression in contractile function that was evident by a marked increase in LVEDP (4.4 fold) with an accompanying 48% decrease in +dP/d*t*, 58% decrease in –dP/d*t*, and 43% decrease in LVSP (Table 10). The contractile function in the infarcted hearts was improved with losartan treatment, as the following parameters illustrated noticeable changes: LVEDP elevation was lowered from 4.4 fold to 1.6 fold, +dP/d*t* increased from 52% to 76%, -dP/d*t* increased from 42% to 76% and LVSP increased from 57% to 81%. No significant alterations in heart rate or MAP were observed among the sham and MI groups with or without drug treatment (Table 10).

Table 10. **Hemodynamic parameters of sham and myocardial infarcted rats with and without losartan treatment for 8 weeks beginning at 12 weeks post coronary artery occlusion.**

Parameter	Sham	MI	Sham + LOS	MI + LOS
Heart rate (bpm)	220 ± 8	230 ± 6	237 ± 3	232 ±11
LVSP (mm Hg)	134 ± 2.5	76 ± 2.1*	125 ± 6	109 ± 4#
LVEDP (mm Hg)	4.7 ± 0.11	20.8 ± 0.70*	5.4 ± 0.63	7.6 ± 0.54#
+dP/dt (mm Hg/s)	7350 ± 400	3827 ± 130*	6529 ± 290	5598 ± 346#
-dP/dt (mm Hg/s)	5620 ± 155	2350 ± 230*	5253 ± 81	4263 ± 250#
MAP (mm Hg)	151 ± 15	135 ± 10	149 ± 13	146 ± 12

Values are mean ± S.E. of 5 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; LOS – Losartan (20 mg/kg/day); *p <0.05 compared with the 20 week sham group; #p < 0.05 compared with the 20 week MI group.

Internal cardiac diastolic and systolic dimensions as measured by echocardiography (Figure 6) showed marked changes in the structure of the heart due to MI (Table 11). The MI hearts showed a 26% decrease in the thickness of IVSs, a 23% increase in LVIDd, a 78% increase in LVIDs, a 55% increase in the thickness of LVPWs, and a 28% increase in the thickness of LVPWd. Attenuation of changes in these parameters was observed with the treatment of losartan, as IVSs increased from 74% to 90%, LVIDs showed a reduction from 178% to 145%, LVPWs showed a reduction of 155% to 107%, and LVPWd showed a decline from 128% to 90%. Interestingly, there was not any significant reduction in the LVIDd value after treatment with losartan. Furthermore, there were no alterations in the thickness of IVSd in any group (Tabel 11). The infarcted hearts showed reductions in cardiac contractile parameters to reflect a 40% decrease in EF, a 50% decrease in FS, and a 54% decline in CO (Table 12). The 8 wks treatment period with losartan revealed improvement of cardiac function, as these values were enhanced with an increase in EF from 60% to 73%, an increase in FS from 50% to 63%, and an increase in CO from 54% to 65%. The heart rate did not show any considerable variation in values. Furthermore, treatment of sham control animals with losartan did not produce any significant changes in parameters for internal cardiac dimensions or cardiac function (Tables 11 and 12).

The SR function correlated with the failure of cardiac contractility as the data in Table 13 show a decrease in Ca^{2+} uptake activity by 68% in MI rat hearts. However, there was an attenuation of this change showing an improvement from 32% to 58% upon losartan treatment. SR Ca^{2+}-release activity was depressed in the untreated MI hearts by 53% but this change was not affected by treatment with losartan (Table 13). MF isolated

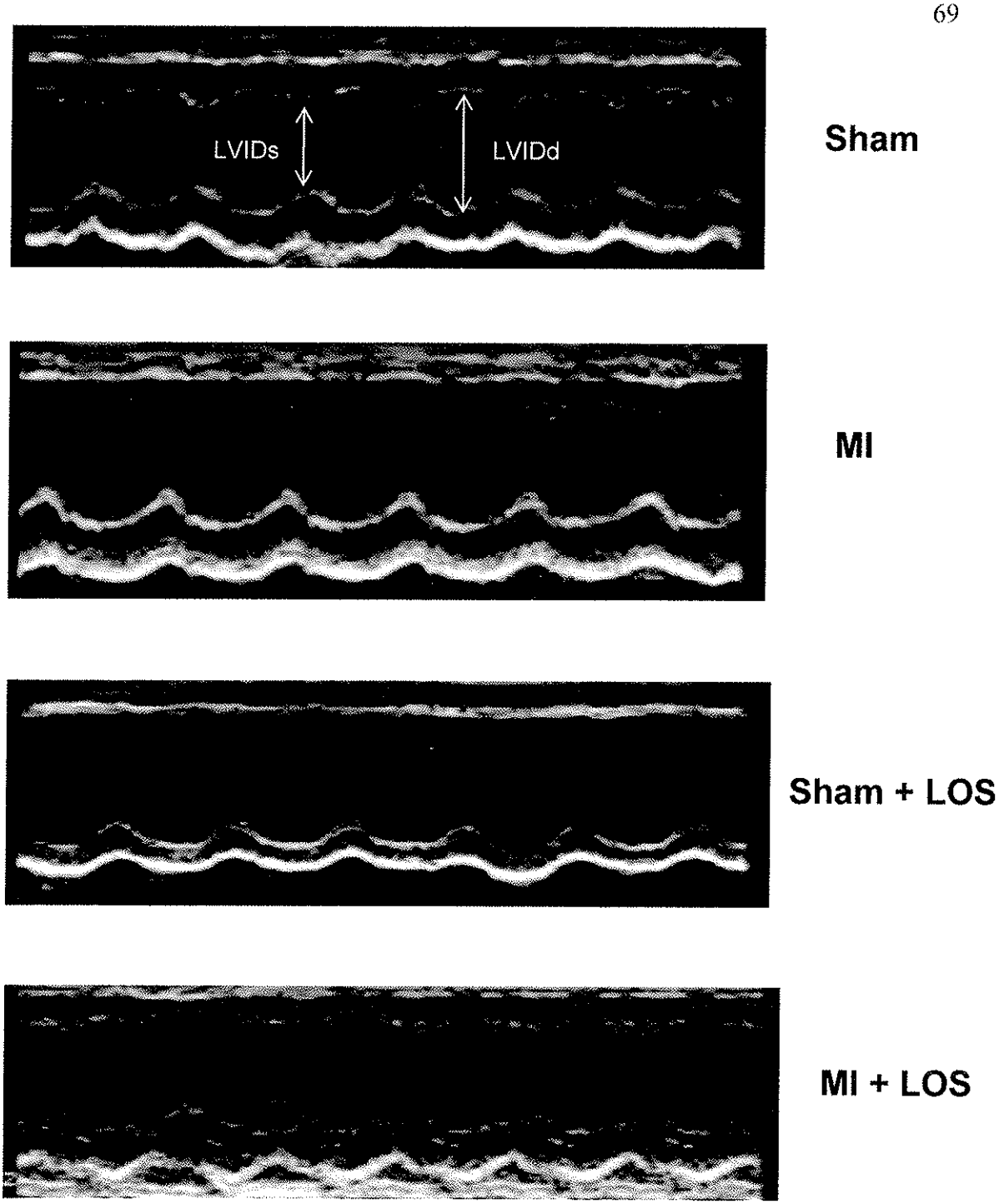


Figure 6. M-Mode echocardiographic images of internal cardiac dimensions of sham and MI rat hearts with and without treatment with losartan (LOS; 20 mg/kg/day) at the time point of 20 weeks post coronary artery occlusion. MI – myocardial infarction; LVID – left ventricular internal diameter; d-diastole; s-systole.

Table 11. **Measurements of internal cardiac diastolic and systolic dimensions by echocardiography of sham and infarcted animals with and without losartan treatment for 8 weeks.**

Parameter	Sham	MI	Sham + LOS	MI + LOS
IVSd (cm)	0.220 ± 0.01	0.227 ± 0.02	0.254 ± 0.03	0.239 ± 0.03
IVSs (cm)	0.387 ± 0.02	0.287 ± 0.01*	0.387 ± 0.03	0.351 ± 0.02#
LVIDd (cm)	0.878 ± 0.03	1.080 ± 0.02*	0.845 ± 0.03	1.060 ± 0.07
LVIDs (cm)	0.468 ± 0.04	0.834 ± 0.05*	0.533 ± 0.04	0.681 ± 0.02#
LVPWs (cm)	0.241 ± 0.02	0.374 ± 0.03*	0.270 ± 0.03	0.259 ± 0.01#
LVPWd (cm)	0.346 ± 0.02	0.444 ± 0.03*	0.305 ± 0.03	0.313 ± 0.03#

Values are mean ± SE of 7 animals in each group. MI – myocardial infarction; LOS – Losartan (20 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 12. **Assessment of cardiac function by echocardiography of sham and infarcted animals with and without losartan treatment for 8 weeks.**

Parameter	Sham	MI	Sham + LOS	MI + LOS
Ejection fraction (%)	80 ± 5.1	48 ± 3.2*	70 ± 3.8	58 ± 2.2#
Fractional shortening (%)	46 ± 3.5	23 ± 1.3*	37 ± 2.7	29 ± 1.4#
Cardiac output (L/min)	0.461± 0.04	0.255± 0.02*	0.457 ± 0.04	0.301 ± 0.04#
Heart rate (bpm)	345 ± 10	360 ± 11	318 ± 7	338 ± 14

Values are mean ± SE of 7 animals in each group. MI – myocardial infarction; LOS – losartan (20 mg/kg/day); bpm – beats per minute; *$p < 0.05$ compared with the 20 week sham group. #$p < 0.05$ compared with the 20 week MI group.

from the MI hearts showed a 39% decrease in MF Ca^{2+}-stimulated ATPase activity without any changes in Mg^{2+}-ATPase activity when compared to the 20 wks sham hearts (Table 13). Treatment of MI animals with losartan, showed a significant reversal from 61% to 79% in MF Ca^{2+}-stimulated ATPase activity. There were no alterations observed in SR Ca^{2+}-transport and MF ATPase activities in control rats upon treatment with SR Ca^{2+}-transport and losartan (Table 13).

c. Alteration in SR and MHC mRNA expression

To understand the mechanisms of changes in subcellular activities in the failing and drug-treated rat hearts, the steady-state mRNA levels for some subcellular proteins were examined using Northern blot analysis (Figures 7 and 8). The failing hearts following MI showed a reduction in mRNA levels for SR proteins with a 21% decline in SERCA2a (Figure 7, panel A), and a 22% decline in PLB (Figure 7, panel B). However, there was no alteration in the mRNA level for RyR (Figure 8, panel B). These changes were partially reversed by losartan treatment, as shown by improvement in the levels of SERCA2a from 79% to 88%, and of PLB from 78% to 99% when compared to the control values of the sham rats, although these effects were not statistically signficant. A significant 39% reduction in the α-MHC mRNA was observed in the MI hearts (Figure 8, panel C), whereas an increase in the β-MHC mRNA level of 200% was noted (Figure 8, panel D). Treatment with losartan was able to attenuate the changes in the mRNA levels for MHC as β-MHC decreased from 300% to 212% whereas that for α-MHC increased from 61% to 77% of the control hearts.

d. Modification of SR protein expression

In order to test if changes in SR Ca^{2+}-uptake activity in the failing heart are

Table 13. **Calcium and magnesium activity biochemical measurements of sham and infarcted animals with and without losartan treatment for 8 weeks.**

	Parameter	Sham	MI	Sham + LOS	MI + LOS
A)	**Sarcoplasmic reticulum activity**				
	Ca^{2+} uptake (nmol Ca^{2+}/mg/min)	53.1 ± 2.01	17.3 ± 2.70*	51.9 ± 3.03	30.6 ± 3.30#
	Ca^{2+} release (nmol Ca^{2+}/mg/15 sec)	8.9 ± 0.20	4.2 ± 0.15*	8.4 ± 0.09	4.3 ± 0.11
B)	**Myofibrillar ATPase activity**				
	Mg^{2+}ATPase (μmol Pi/mg/hr)	3.1 ± 0.07	3.0 ± 0.10	3.2 ± 0.12	2.9 ± 0.11
	Ca^{2+} stimulated ATPase (μmol Pi/mg/hr)	13.2 ± 0.80	8.1 ± 0.61*	12.9 ± 0.73	10.5 ± 0.50#

Values are mean ± SE of 7 animals in each group. MI – myocardial infarction; LOS – Losartan (20 mg/kg/day); * $p < 0.05$ compared with the 20 week sham group; # $p < 0.05$ compared with the 20 week MI group.

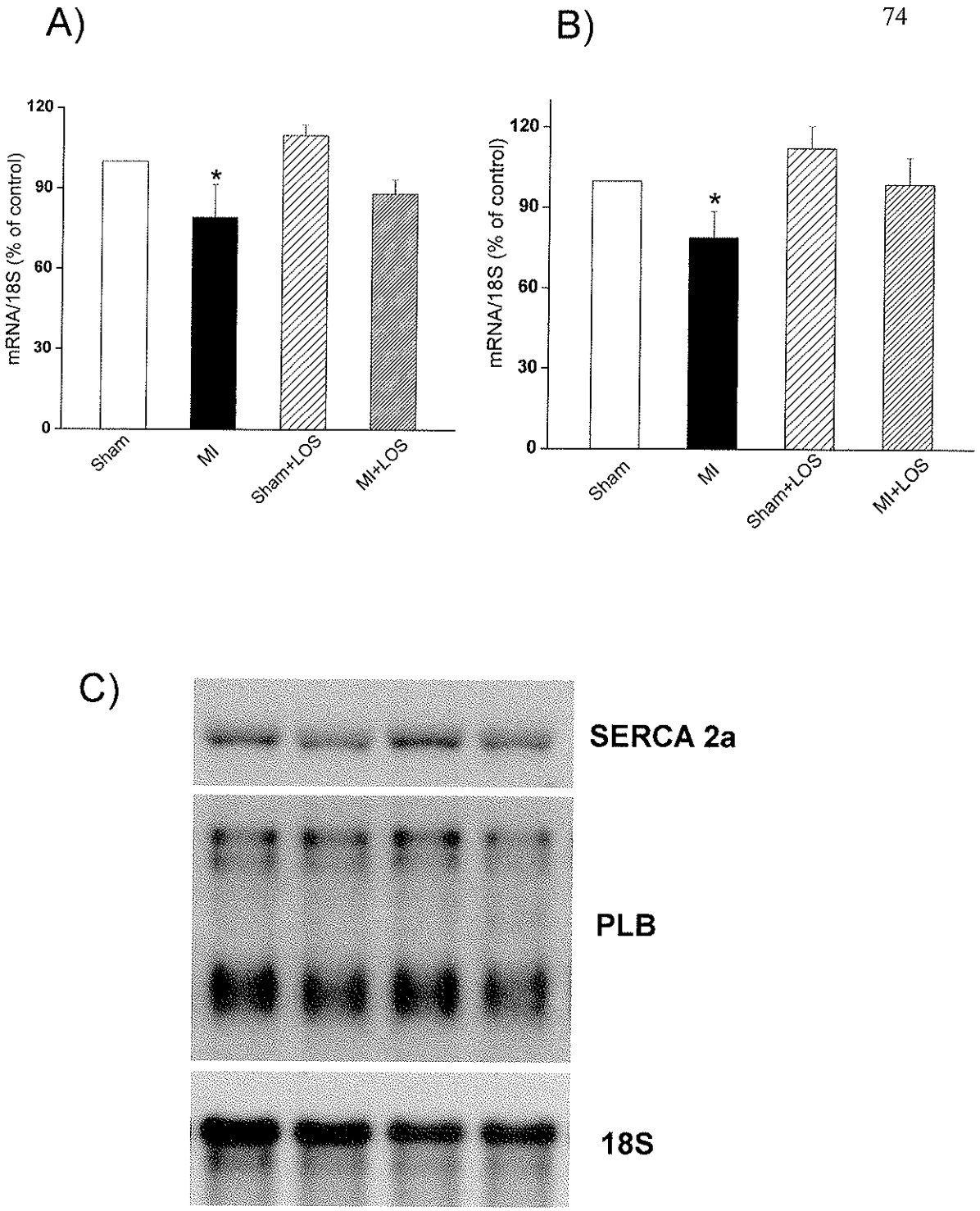


Figure 7. Relative mRNA levels for sarcoplasmic reticular (SR) sarcoplasmic reticulum Ca^{2+}-pump ATPase (SERCA2a, panel A), and phospholamban (PLB, panel B), as well as their corresponding Northern blots (panel C) from sham and infarcted (MI) rats, with or without losartan (LOS) treatment. *$p < 0.05$ compared with sham.

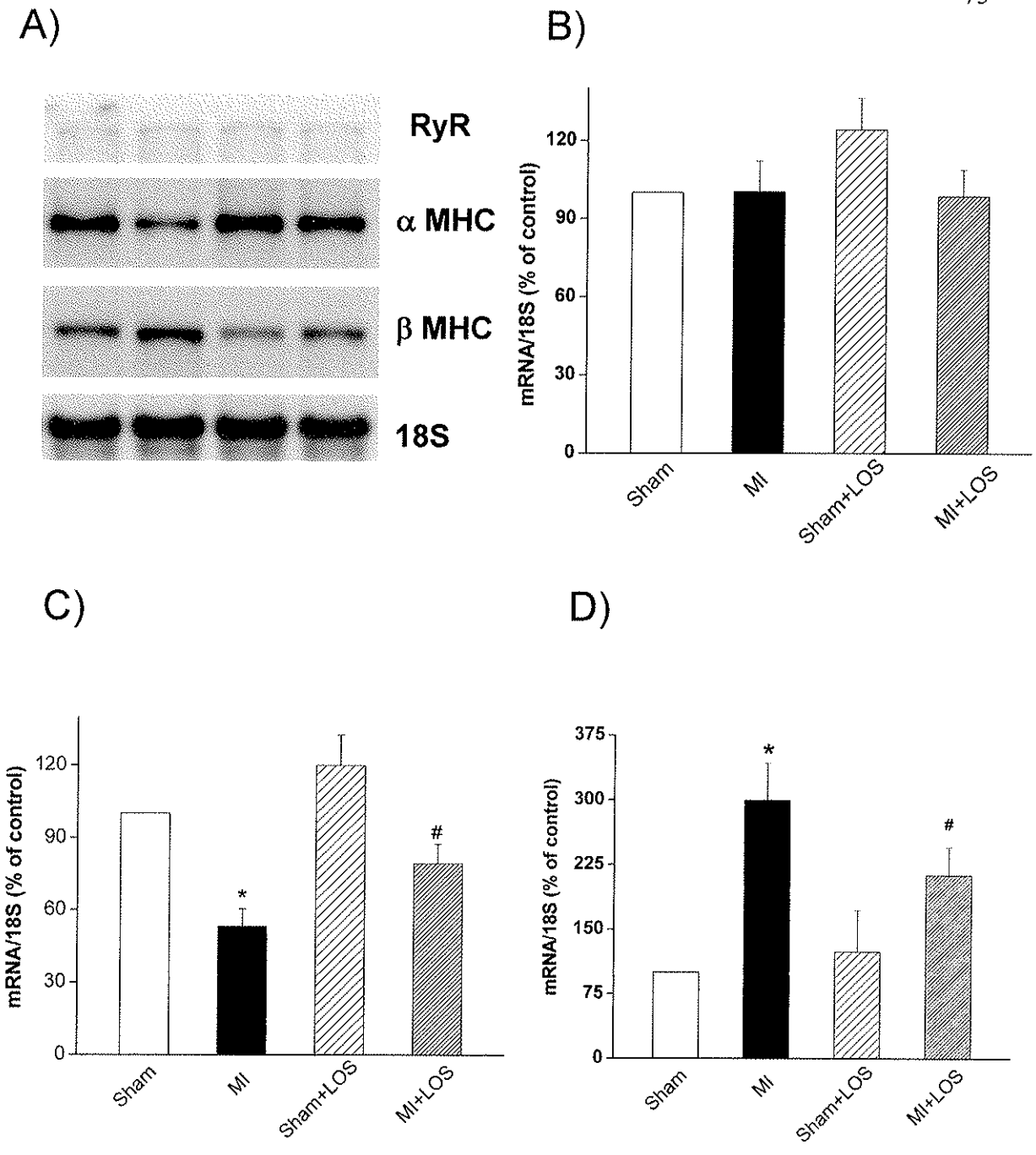


Figure 8. Typical Northern blots (panel A) and relative mRNA levels for sarcoplasmic reticular ryanodine receptor (RyR, panel B), alpha myosin heavy chain (αMHC, panel C), and beta myosin heavy chain (βMHC, panel D) isozymes from sham and infarcted (MI) rat hearts with or without losartan (LOS) treatment. *$p < 0.05$ compared with sham. #$p < 0.05$ compared with MI group .

associated with alterations in the expression of Ca^{2+}-cycling and regulatory proteins, content for SR PLB, SERCA2a, and CQS were measured (Figure 9). The failing heart showed a reduction in the expression of PLB by 41% (Figure 9, panel B) and SERCA2a by 68% (Figure 9, panel C), without any significant decrease in the protein expression of CQS (Figure 9, panel D). Upon the treatment of MI animals with losartan, these hearts revealed a significant amount of recovery in protein expression as values increased from 59% to 71% for PLB, 32% to 74% for SERCA2a, and no apparent change in the values for CQS. Treatment of control animals with losartan had no effect on SR protein content.

e. Alteration in Plasma catecholamine levels

Plasma NE and E were substantially higher in the infarcted rats as compared to the sham rats (1.9 fold increase and 1.7 fold increase, respectively; Table 14). Treatment of MI animals with losartan showed a further increase in the circulation levels of NE (1.3 fold increase), without any change in E levels. Plasma dopamine levels were markedly elevated in MI rats (3.1 fold increase); however, treatment with losartan showed a significant reduction (from 203 to 79 pg/ml) in dopamine levels in the infarcted rats. Treatment of control animals with losartan showed no significant effect on plasma levels of catecholamines.

3. Effects of β-AR Blockade on MI Rat Hearts due to CHF

a. Attenuation of changes in general characteristics due to MI

The occurrence of cardiac remodeling post MI was associated with the presence of a large scar and ventricular chamber hypertrophy, as mentioned previously. Treatment of

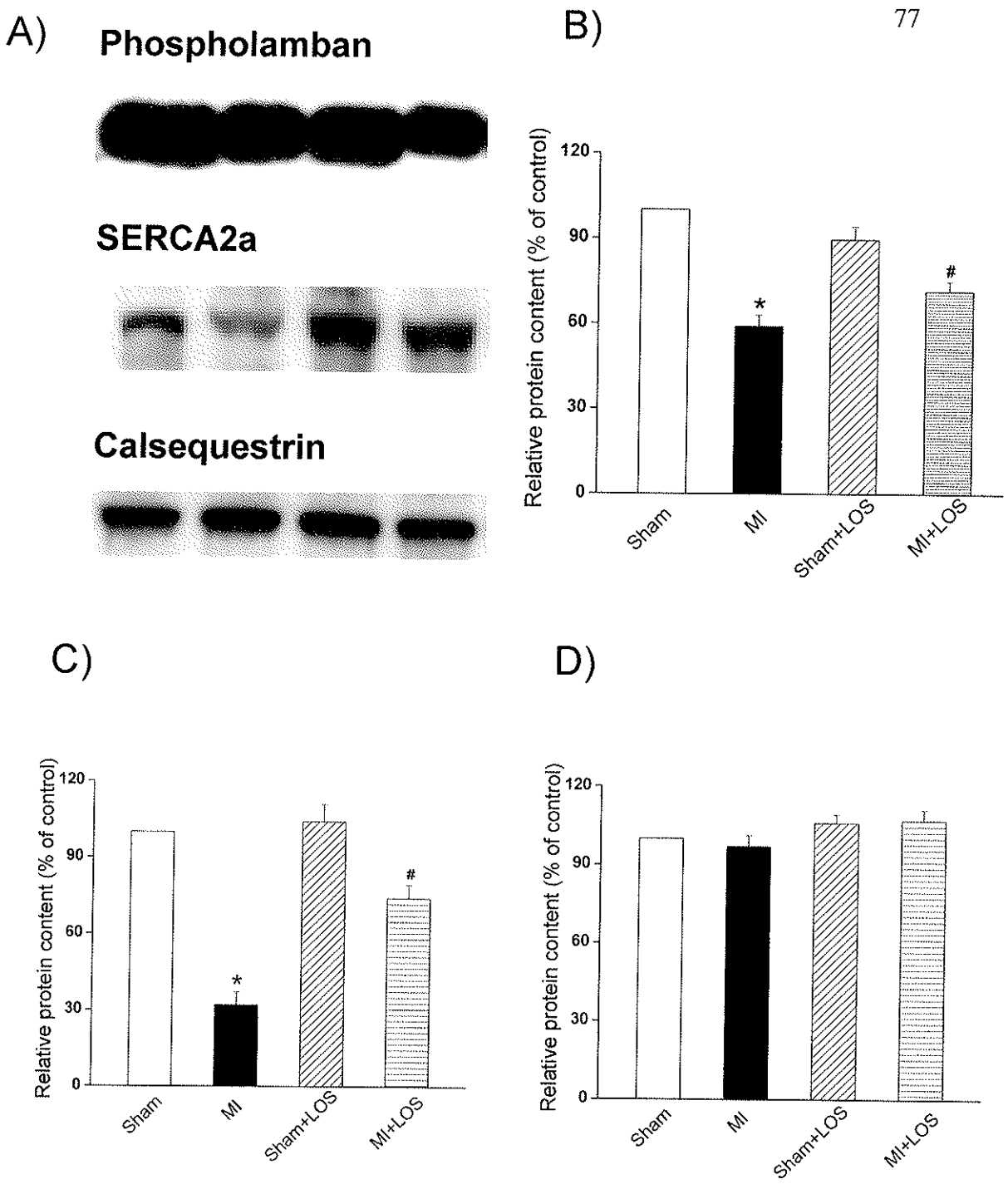


Figure 9. Typical Western blots (panel A) and relative protein content of sarcoplasmic reticular phospholamban (PLB, panel B), Ca^{2+}-pump (SERCA2a, panel C), and calsequestrin (CQS, panel D) from sham and infarcted (MI) rat hearts with or without losartan (LOS) treatment. *$p < 0.05$ compared with sham. #$p < 0.05$ compared with MI group.

Table 14. **Measurement of plasma concentration catecholamine levels of rats with and without losartan treatment at 20 weeks post coronary artery occlusion.**

Parameter	Sham	MI	Sham + LOS	MI + LOS
Norepinephrine (pg/ml)	182 ± 4.8	355 ± 13.2 *	197 ± 6.0	455 ± 15 #
Epinephrine (pg/ml)	78 ± 7.0	132 ± 5.6 *	77 ± 6.2	131 ± 4.5
Dopamine (pg/ml)	66 ± 7.3	203 ± 19.7 *	77 ± 4.5	79 ± 5.0 #

Values are mean ± SE of 5 animals in each group. MI – myocardial infarction; LOS – Losartan (20 mg/kg.day); *$p < 0.05$ compared with the 20 week sham group. #$p < 0.05$ compared with the 20 week MI group.

the MI animals with a β_1-AR antagonist, metoprolol, showed an attenuation of the increase in heart wt from 54% to 27% as compared to control hearts (Table 15). The heart wt/body wt ratio was also attenuated with metoprolol from 56% increase to 29% increase. The RV wt showed a significant (64%) increase due to MI which was corrected with metoprolol to a 15% increase as compared to sham hearts. Metaprolol treatment also attenuated the elevation in the lung wet/dry ratio from 33% increase to 7% increase as compared to the control group, but showed not affect on the liver wet/dry ratio in MI animals. Treatment of control animals with metaprolol did not affect the general characteristics (Table 15).

b. Cardiac performance, subcellular activities, and ventricular remodeling

The 20 wks post MI hearts showed a reduction in cardiac function that was apparent by a significant elevation in LVEDP (4.6 fold) and with a corresponding 48% decrease in +dP/d*t*, 57% decrease in –dP/d*t*, and a 41% decrease in LVSP (Table 16). The cardiac function in MI animals was improved with metoprolol treatment, as the following parameters showed considerable changes: LVEDP elevation was lowered from 4.6 fold to 1.7 fold, +dP/d*t* increased from 52% to 67%, and -dP/d*t* increased from 43% to 68%. Treatment of control animals with metoprolol had no significant effect on any of the cardiac performance parameters (Table 16). Furthermore, no significant changes in heart rate or MAP were detected among untreated and metoprolol treated groups.

Internal cardiac diastolic and systolic echocardiographic measurements (Table 17) indicated that there occurred 24% decrease in IVSs, 18% increase in LVIDd, 79% increase in LVIDs, 52% increase in LVPWs, and 34% increase in LVPWd in the failing hearts. Metoprolol treatment of MI animals showed reversal of

Table 15. **General characteristics of sham and infarcted rats with and without metoprolol treatment for 8 weeks starting at 12 weeks after coronary artery occlusion.**

Parameter	Sham	MI	Sham + MET	MI + MET
Body wt (g)	682 ± 32	674 ± 30	651 ± 20	617 ± 13
Heart wt (mg)	1783 ± 65	2750 ± 80*	1825 ± 15	2278 ± 12#
Scar wt (mg)	ND	778 ± 137	ND	540 ± 68
Scar wt/LV wt (%)	ND	43 ± 5	ND	42 ± 8
Heart wt/body wt (mg/g)	2.7 ± 0.09	4.3 ± 0.18*	2.9 ± 0.16	3.4 ± 0.13#
RV wt (mg)	383 ± 30	629 ± 80*	267 ± 33	443 ± 20#
Lung wet/dry wt ratio	3.9 ± 0.09	5.3 ± 0.23*	4.1 ± 0.13	4.3 ± 0.10#
Liver wet/dry wt ratio	2.7 ± 0.13	2.9 ± 0.06	2.9 ± 0.03	2.9 ± 0.06

Values are mean ± SE of 6 animals in each group. MI – myocardial infarction; MET – Metoprolol (50 mg/kg/day); ND – not detected; wt – weight; LV – left ventricle; RV – right ventricle;*$p < 0.05$ compared with the 20 weeks sham group; #$p < 0.05$ compared with the 20 week MI group.

Table 16. **Hemodynamic parameters of sham and myocardial infarcted rats with and without metoprolol treatment for 8 weeks beginning at 12 weeks post coronary artery occlusion.**

Parameter	Sham	MI	Sham + MET	MI + MET
Heart rate (bpm)	228 ± 11	234 ± 9	230 ± 10	225 ± 10
LVSP (mm Hg)	132 ± 2	78 ± 2*	135 ± 5	115 ± 6#
LVEDP (mm Hg)	4.5 ± 0.50	21.0 ± 0.81*	3.9 ± 0.86	7.8 ± 0.33#
+dP/dt (mm Hg/s)	7394 ± 409	3856 ± 145*	6669 ± 367	4963 ± 357#
-dP/dt (mm Hg/s)	5609 ± 164	2390 ± 249*	5123 ± 161	3793 ± 367#
MAP (mm Hg)	161 ± 14	130 ± 20	165 ± 13	147 ± 14

Values are mean ± SE of 7 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; MET – Metoprolol (50 mg/kg/day);
*p <0.05 compared with the 20 week sham group; #p < 0.05 compared with the 20 week MI group.

these aspects of cardiac remodeling as IVSs increased from 76% to 97%, LVIDs showed a reduction from 179% to 136%, LVPWs showed a reduction of 152% to 106%, and LVPWd showed a decline from 134% to 95%. Interestingly, there was not a significant reduction in the LVIDd value upon treatment of MI animals with metoprolol. In addition, there were no significant alterations in IVSd among any groups of animals and there was no effect on various parameters of cardiac remodeling by metoprolol in control animals. The M-Mode echocardiographic (Figure 10) examination revealed 53% decrease in EF, 52% decrease in FS%, and 56% decline in the CO in MI rats (Table 18). The treatment of MI animals with metoprolol enhanced cardiac function, as different parameters of cardiac performance were increased; EF from 48% to 75%, FS from 48% to 79%, and CO from 44% to 66% without any changes in heart rate. Treatment of control animals with metoprolol did not affect any parameter of cardiac performance significantly (Table 18).

Both SR Ca^{2+}-uptake and Ca^{2+}-release activities were determined in control and MI hearts with or without metoprolol treatment. Table 19 shows a decline in Ca^{2+} uptake in MI hearts with a 76% reduction in activity whereas Ca^{2+}-release activity was depressed by 53%. Upon β-blocker treatment however, there was an improvement from 24% to 45% in Ca^{2+}-uptake activity without any changes in Ca^{2+}-release activity. MF from the viable LV of the MI rat hearts showed a decreased Ca^{2+}-stimulated ATPase activity of 40% when compared to the 20 wks sham hearts (Table 19). Treatment with metoprolol did not show any significant reversal of the depressed MF Ca^{2+}-stimulated ATPase activity. Moreover, there were no alterations observed in the levels of MF Mg^{2+}-ATPase activity in the control and drug-treated groups (Table 19). Treatment of control animals with metoprolol showed no effect on SR Ca^{2+}-transport and MF ATPase activities (Table

Table 17. **Measurements of internal cardiac diastolic and systolic dimensions by echocardiography of sham and infarcted animals with and without metoprolol treatment for 8 weeks.**

Parameter	Sham	MI	Sham + MET	MI + MET
IVSd (cm)	0.200 ± 0.02	0.230 ± 0.01	0.251 ± 0.01	0.283 ± 0.02
IVSs (cm)	0.380 ± 0.03	0.290 ± 0.02*	0.392 ± 0.02	0.370 ± 0.03#
LVIDd (cm)	0.869 ± 0.02	1.030 ± 0.03*	0.826 ± 0.04	1.030 ± 0.05
LVIDs (cm)	0.473 ± 0.02	0.850 ± 0.05*	0.489 ± 0.03	0.645 ± 0.05#
LVPWs (cm)	0.250 ± 0.02	0.380 ± 0.01*	0.265 ± 0.02	0.267 ± 0.02#
LVPWd (cm)	0.343 ± 0.02	0.459 ± 0.03*	0.315 ± 0.02	0.327 ± 0.01#

Values are mean ± SE of 7 animals in each group. MI – Myocardial infarction; MET – Metoprolol (50 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.

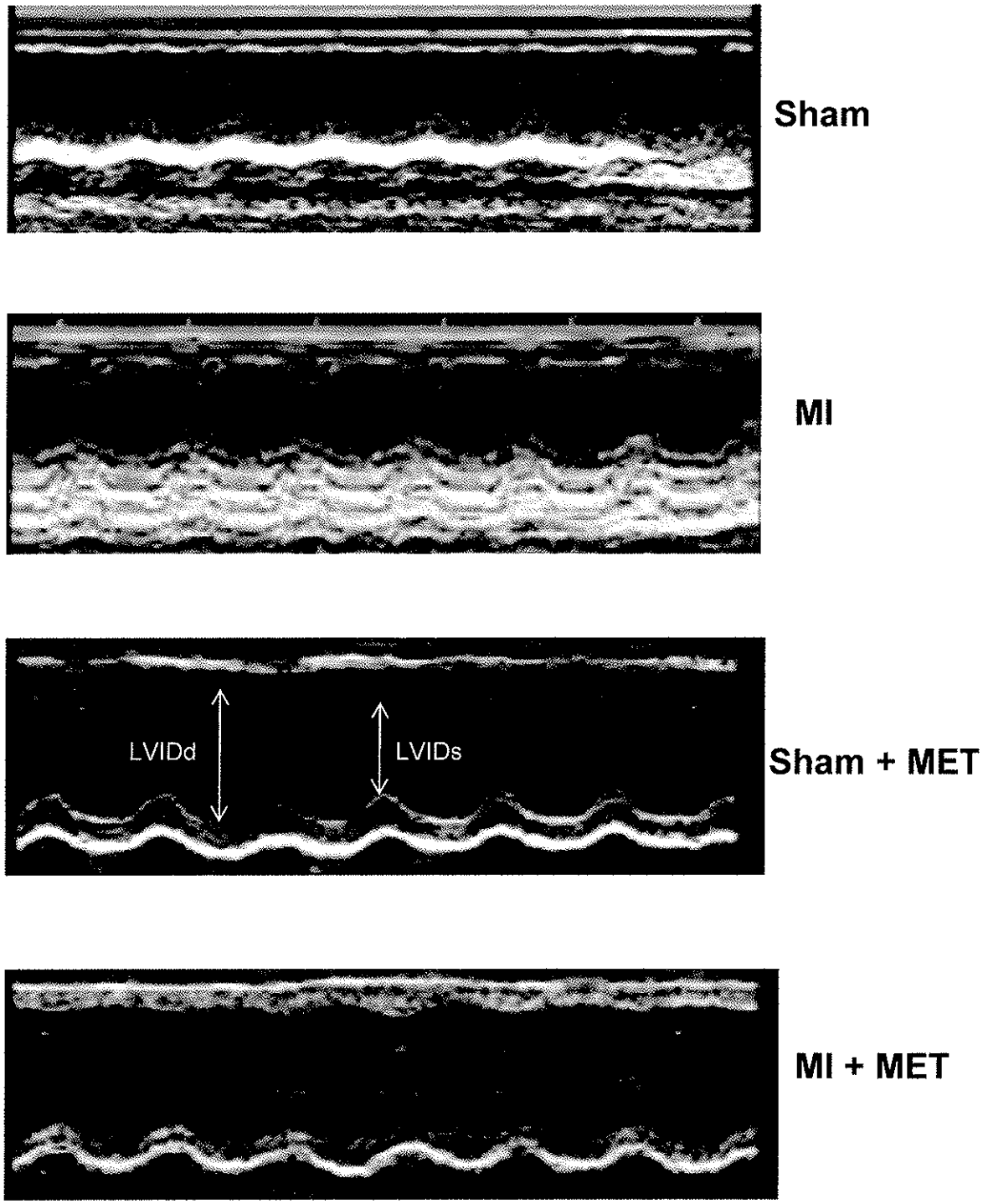


Figure 10. M-Mode echocardiographic images of internal cardiac dimensions of sham and MI rat hearts with and without treatment with Metoprolol (MET; 50 mg/kg/day) at the time point of 20 weeks post coronary artery occlusion; MI – myocardial infarction; LVID – left ventricular internal diameter; d-diastole; s-systole.

Table 18. **Assessment of cardiac function by echocardiography of sham and infarcted animals with and without metoprolol treatment for 8 weeks.**

Parameter	Sham	MI	Sham + MET	MI + MET
Ejection fraction (%)	90 ± 4.0	43 ± 3.0*	76 ± 2.0	68 ± 5.0#
Fractional shortening (%)	45 ± 4.1	22 ± 1.7*	41 ± 1.7	36 ± 3.9#
Cardiac output (L/min)	0.493 ± 0.02	0.221 ± 0.03*	0.373 ± 0.02	0.325 ± 0.05#
Heart rate (bpm)	335 ± 10	346 ± 10	320 ± 13	345 ± 15

Values are mean ± SE of 7 animals in each group. MI – myocardial infarction; MET – Metoprolol (50 mg/kg/day); bpm- beats per minute; *$p < 0.05$ compared with the 20 week sham group. #$p < 0.05$ compared with the 20 week MI group.

19).

c. Alteration in SR and MHC mRNA expression

In order to examine the effects of metoprolol on gene expression for some SR and MF proteins, the steady-state mRNA levels for SR and MF proteins were monitored using the Northern blot analysis (Figures 11 and 12). The infarcted rat hearts showed a decreased level of mRNA with a 24% reduction in SERCA2a (Figure 11, panel A), and a 42% reduction in PLB (Figure 11, panel B). However, there was no significant modification in the level of mRNA for RyR (Figure 12, panel B). These alterations were not reversed significantly by 8 wks treatment with metoprolol. The α- and β-MHC isozyme mRNA levels were also measured in control and MI hearts with or without drug treatment. A decrease of 42% in α-MHC mRNA level (Figure 12, panel C) in the failing hearts and a marked increase of 261% in β-MHC mRNA level was seen (Figure 12, panel D). Treatment of MI animals with metoprolol was able to reverse β-MHC from 261% to 170% (Figure 12, panel D). On the other hand, treatment of control animals with metoprolol showed no effect on SR and MF gene expression.

d. Modification of SR protein expression

To gain information regarding alterations in SR Ca^{2+}-uptake activity in the failing hearts, protein content of SR PLB, SERCA2a, and CQS were examined in control and MI hearts (Figure 13). The infarcted hearts showed an appreciable decline in the expression of PLB levels by 42% (Figure 13, panel B), SERCA2a by 70% (Figure 13, panel C) without any significant decrease in the protein levels of CQS (Figure 13, panel D). After therapy with metoprolol, the MI hearts exhibited a considerable amount of recovery in the protein levels as evidenced by an elevation from 55% to 71% for PLB, 30% to 78%

Table 19. **SR Ca^{2+}-transport and MF ATPase activities of sham and infarcted animals with and without metoprolol treatment for 8 weeks.**

	Parameter	Sham	MI	Sham + MET	MI + MET
A)	**Sarcoplasmic reticulum activity**				
	Ca^{2+} uptake (nmol Ca^{2+}/mg/min)	58.7 ± 3.21	14.0 ± 2.50*	49.1 ± 3.02	26.2 ± 1.22#
	Ca^{2+} release (nmol Ca^{2+}/mg/15 sec)	8.6 ± 0.35	4.0 ± 0.70*	8.3 ± 0.56	4.2 ± 0.69
B)	**Myofibrillar ATPase activity**				
	Mg^{2+}ATPase (μmol Pi/mg/hr)	3.4 ± 0.21	3.6 ± 0.15	3.5 ± 0.23	3.7 ± 0.29
	Ca^{2+} stimulated ATPase (μmol Pi/mg/hr)	12.9 ± 0.68	7.8 ± 0.54*	12.7 ± 0.67	8.0 ± 0.46

Values are mean ± SE of 7 animals in each group. MI – myocardial infarction; MET – Metoprolol (50 mg/kg/day); * $p < 0.05$ compared with the 20 week sham group; # $p < 0.05$ compared with the 20 week MI group.

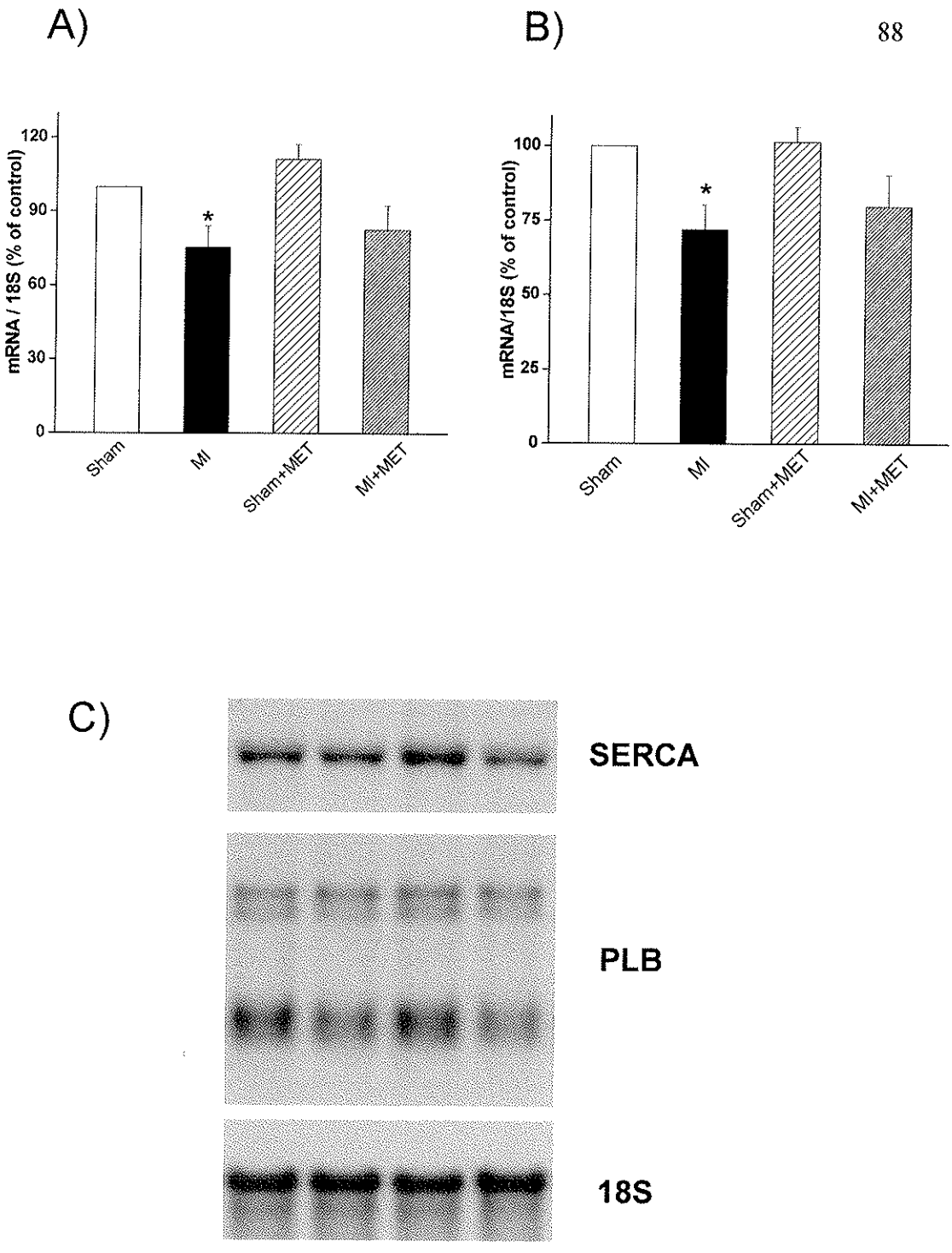


Figure 11. Relative mRNA contents of sarcoplasmic reticular (SR) Ca^{2+}- stimulated ATPase (SERCA2a, panel A), and phospholamban (PLB, panel B), as well as their corresponding Northern blots (panel C) from sham and infarcted (MI) rat hearts with or without treatment. MET - metoprolol; *$p < 0.05$ compared with sham group.-

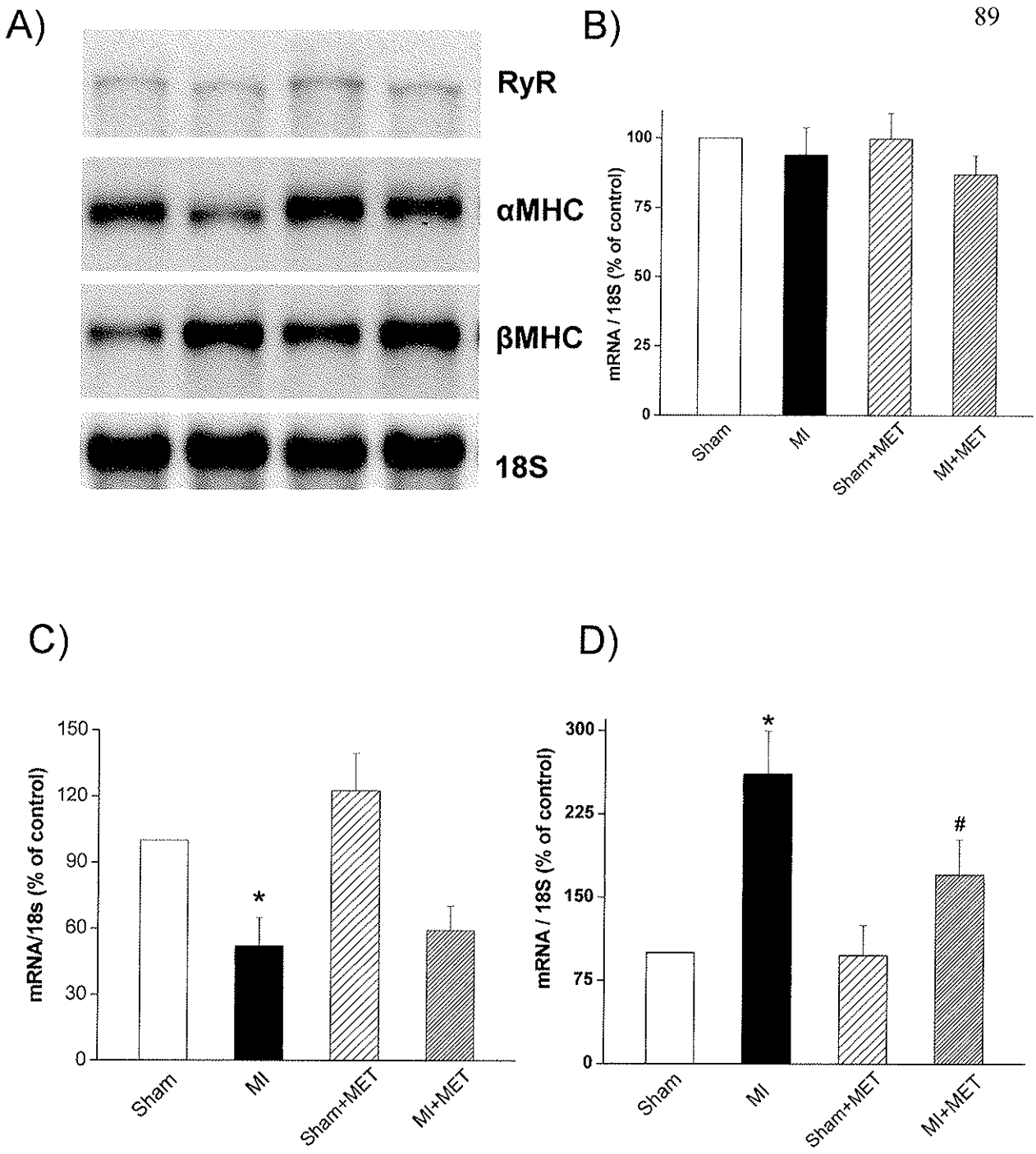


Figure 12. Typical Northern blots (panel A) and relative mRNA contents of sarcoplasmic reticular ryanodine receptor (RyR, panel B),alpha myosin heavy chain (αMHC, panel C), and beta myosin heavy chain (βMHC, panel D) isozymes from sham and infarcted (MI) rat hearts with or without treatment. MET - Metoprolol; *$p < 0.05$ compared with sham. #$p < 0.05$ compared with MI group .

for SERCA2a, without any apparent change in the values for CQS. Treatment of control animals with metoprolol had no effect on SR protein content.

e. Alteration in Plasma catecholamine levels

Plasma NE and E were significantly elevated in the infarcted rats as compared to the sham rats (1.9 fold increase and 1.7 fold increase, respectively; Table 20). Treatment of MI animals with metoprolol revealed a considerable reduction in the circulating levels of NE (0.68 fold decrease), without any significant decrease in E levels. Plasma dopamine levels in MI animals were also elevated (3.2 fold increase), and treatment with metoprolol showed a significant reduction (0.6 fold decrease) in dopamine levels in the infarcted rats (Table 20).

4. Effects of α-AR Blockade on MI Rat Hearts due to CHF

a. Attenuation of General characteristics due to MI

It can be seen from Table 21 that MI for a period of 20 wks was found to produce 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 MI animals for 8 wks with an α_1-AR antagonist, prazosin, revealed a reduction in heart wt from an increase of 56%, to 34% when compared to control hearts (Table 21). The heart wt/body wt ratio was also partially reversed with prazosin from 54% increase to 27% increase. A marked (63%) increase in RV was reversed by prazosin to an 8% increase whereas the increase in lung wet/dry ratio decreased from 30% to 1% below the value of control group. However, prazosin treatment did not affect the liver wet/dry ratio. Treatment of control animals with prazosin had no effect any parameter of the general characteristics (Table 21).

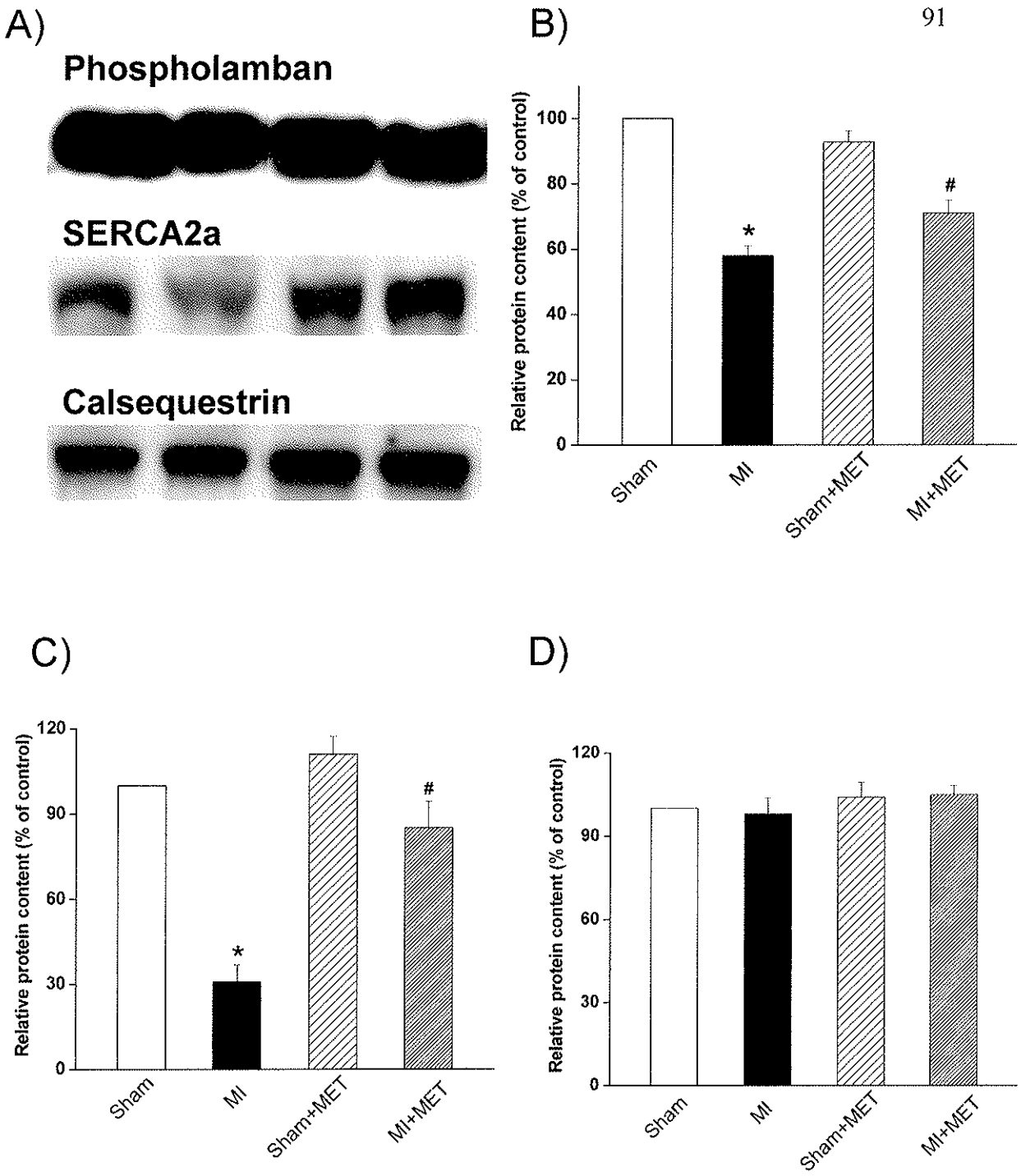


Figure 13. Typical Western blots (panel A) and relative protein content of sarcoplasmic reticular (SR) phospholamban (PLB, panel B), Ca^{2+}-pump ATPase (SERCA2a panel C), and calsequestrin (CQS, panel D) from sham and infarcted (MI) rat hearts with or without treatment. MET - Metoprolol; *$p < 0.05$ compared with sham. #$p < 0.05$ compared with MI group.

Table 20. **Plasma concentration catecholamine levels of rats with and without metoprolol treatment at 20 weeks post coronary artery occlusion.**

Parameter	Sham	MI	Sham + MET	MI + MET
Norepinephrine (pg/ml)	181 ± 5.1	350 ± 10.3 *	170 ± 9.8	240 ± 14.6 #
Epinephrine (pg/ml)	77 ± 6.0	134 ± 7.1 *	81 ± 5.1	128 ± 7.5
Dopamine (pg/ml)	64 ± 9.3	202 ± 11.4 *	78 ± 8.5	118 ± 9.0 #

Values are mean ± SE of 5 animals in each group. MI – myocardial infarction; MET - Metoprolol (50 mg/kg/day); *$p < 0.05$ compared with the 20 week sham group. #$p < 0.05$ compared with the 20 week MI group.

Table 21. **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
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; wt – weight; 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.

b. Cardiac performance, subcellular activities and ventricular remodeling

The failing rat hearts exhibited a 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 22). An improvement in cardiac function in the failing hearts was observed with prazosin treatment as 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 of control animals with prazosin showed no action on cardiac performance.

Table 23 shows the internal cardiac diastolic and systolic echocardiographic measurements of the failing rat hearts to illustrate a significant 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 and there were no alterations observed with the IVSd measurement. Treatment of control animals with prazosin showed no effect on any parameter for cardiac remodeling. To broaden our understanding of the changes involved in the decline of cardiac function, further studies with M-Mode echocardiography were employed (Figure 14). It was observed that the 20 wks post MI rat hearts showed reductions in the fundamental cardiac parameters with 49% decrease in EF, 53% decrease in FS%, and 52% decline in CO (Table 24). Treatment of MI animals with prazosin revealed partial

Table 22. **Hemodynamic parameters of 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
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

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 23. **Internal cardiac diastolic and systolic dimensions as measured by echocardiography of sham and infarcted animals with and without prazosin treatment for 8 weeks**

Parameter	Sham	MI	Sham + PRAZ	MI + PRAZ
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#

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.

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%. In agreement with the previous two studies, the heart rate that was measured at that time did not show any considerable variation in values. Likewise, treatment of control animals with prazosin showed no effect on any parameter for cardiac function.

Table 25 shows decline in SR Ca^{2+} uptake and Ca^{2+}-release in 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 25). There were no alterations observed in MF Mg^{2+}-ATPase activity in the infarcted hearts. Treatment of infarcted animals with prazosin did not reveal any effect on altered SR Ca^{2+}-transport and MF Ca^{2+}-stimulated ATPase activities. Similarly, treatment of control animals with prazosin showed no effect on SR or MF activities.

c. Alteration in SR and MHC mRNA expression

The steady-state mRNA levels for the SR MHC proteins were determined by Northern blot analysis (Figures 15 and 16). The MI rat hearts exhibited a decreased level of mRNA for SR proteins with 22% reduction in SERCA2a (Figure 15, panel A), and 24% reduction in PLB (Figure 15, panel B). However, there was no modification in the level of mRNA for RyR (Figure 16, panel B). The α- and β-MHC mRNA levels were also altered in the MI hearts where α-MHC was decreased by 41% (Figure 16, panel C), and β-MHC was increased by 170% (Figure 16, panel D). Treatment of infarcted animals with prazosin did not show any significant changes in mRNA levels for either SR or MF proteins. Likewise, prazosin had no effect in these parameters for sham control hearts

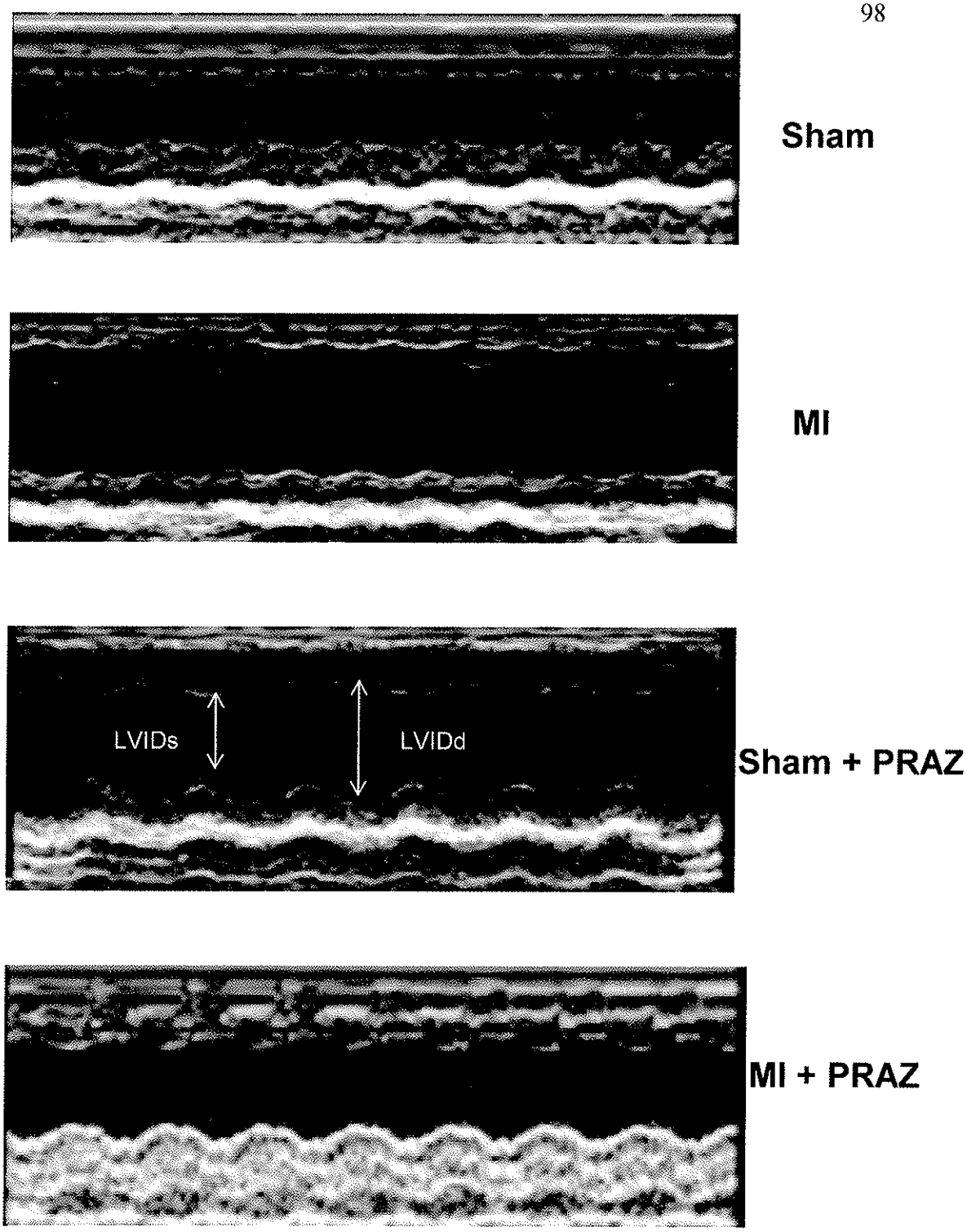


Figure 14. M-Mode echocardiographic images featuring internal cardiac dimensions of sham and MI rat hearts with and without treatment with Prazosin (PRAZ; 10 mg/kg/day) at the time point of 20 weeks post coronary artery occlusion; MI – myocardial infarction; LVID – left ventricular internal diameter; d-diastole; s-systole.

Table 24. **Cardiac function measured by echocardiography of sham and infarcted animals with and without prazosin treatment for 8 weeks.**

Parameter	Sham	MI	Sham + PRAZ	MI + PRAZ
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); bpm – beats per min; *$p < 0.05$ compared with the 20 week sham group. #$p < 0.05$ compared with the 20 week MI group.

Table 25. **SR Ca^{2+}-transport and MF ATPase activities in sham and infarcted animals with and without prazosin treatment for 8 weeks.**

	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*	12.2 ± 0.80	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.

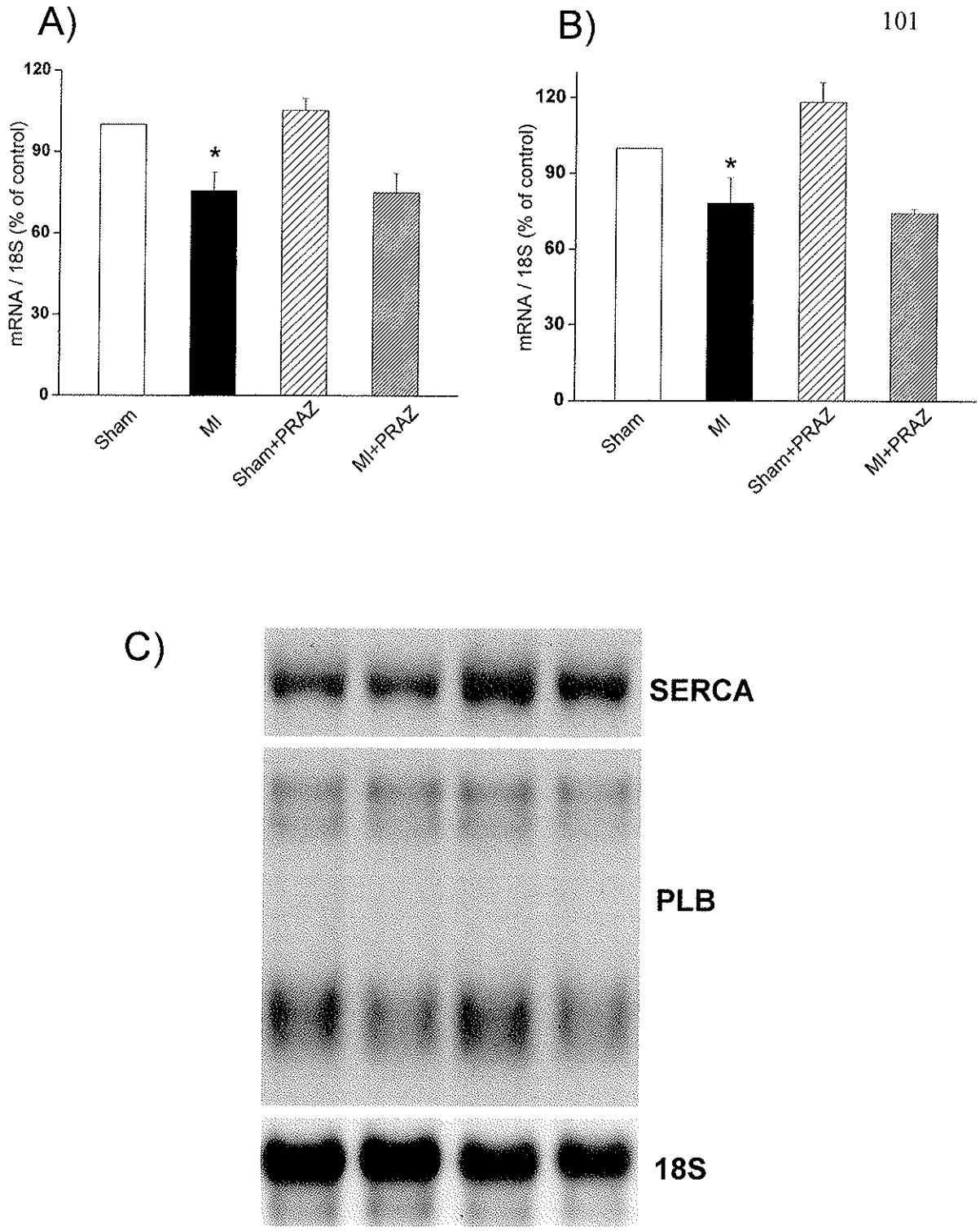


Figure 15. Relative mRNA content of sarcoplasmic reticular (SR) Ca^{2+} -pump ATPase (SERCA2a, panel A), phospholamban (PLB, panel B), and their corresponding Northern blots (panel C) from sham and infarcted (MI) rat hearts with or without treatment. PRAZ - prazosin; *$p < 0.05$ compared with sham.

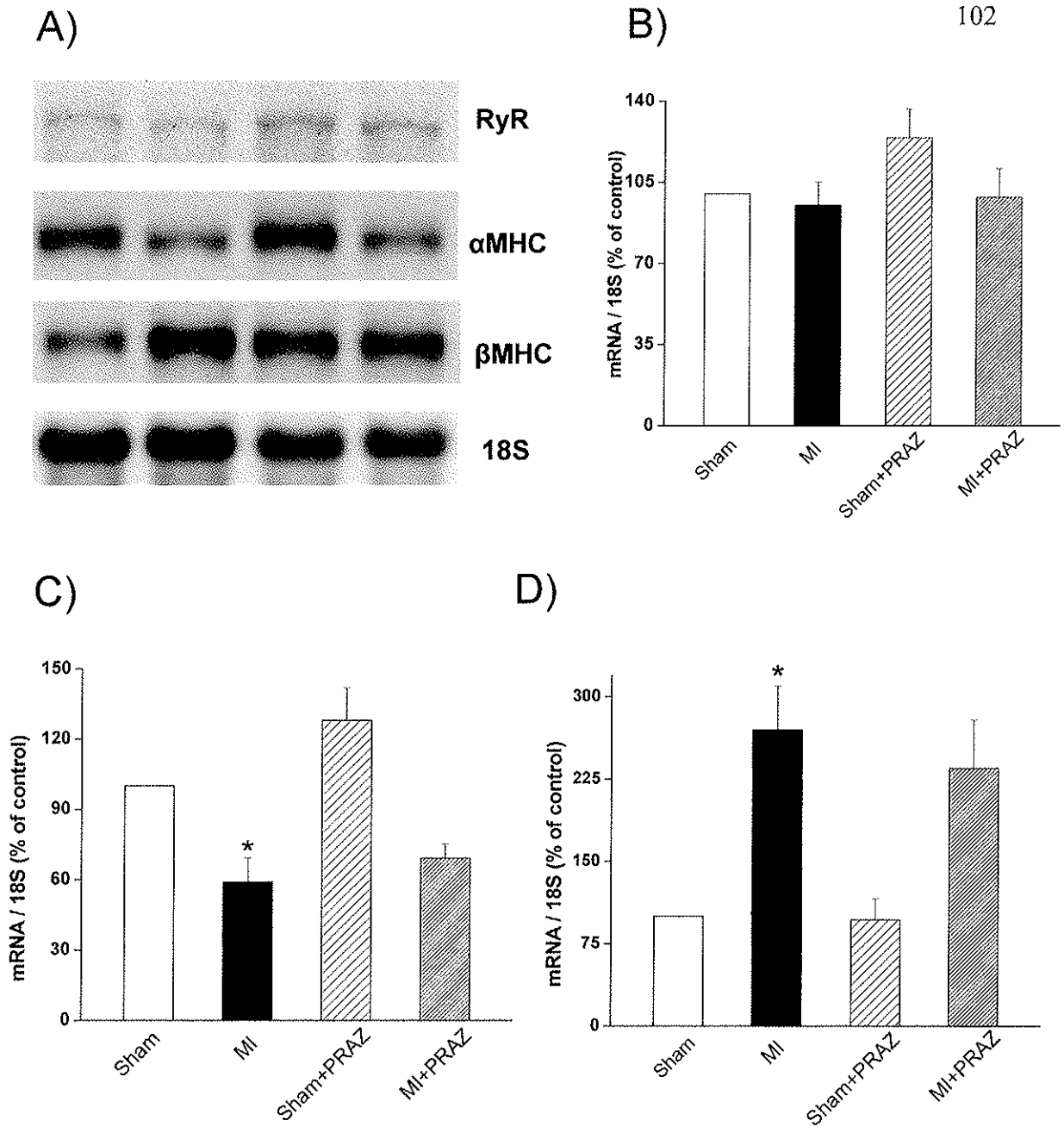


Figure 16. Typical Northern blots (panel A) and relative mRNA contents of sarcoplasmic reticular ryanodine receptor (RyR, panel B), alpha myosin heavy chain (αMHC, panel C), and beta myosin heavy chain (βMHC, panel D) isozymes from sham and infarcted (MI) rat hearts with or without treatment. PRAZ - Prazosin; *p <0.05 compared with sham.

(Figures 15, 16).

d. Modification of SR protein expression

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 17). The MI hearts showed a decline in the expression of PLB by 43% (Figure 17, panel B), SERCA2a by 69% (Figure 17, panel C), without any significant modifications in the protein expression of CQS (Figure 17, panel D). After therapy with prazosin, the infarcted hearts did exhibit a considerable 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. Treatment of control animals with prazosin showed no effect on SR protein expression.

e. Alteration in Plasma catecholamine levels

Plasma NE and E were considerably elevated in the infarcted rats as compared to the sham rats (1.9 fold increase and 1.7 fold increase, respectively, Table 26). 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 26). Treatment of control animals with prazosin had no effect on plasma catecholamines.

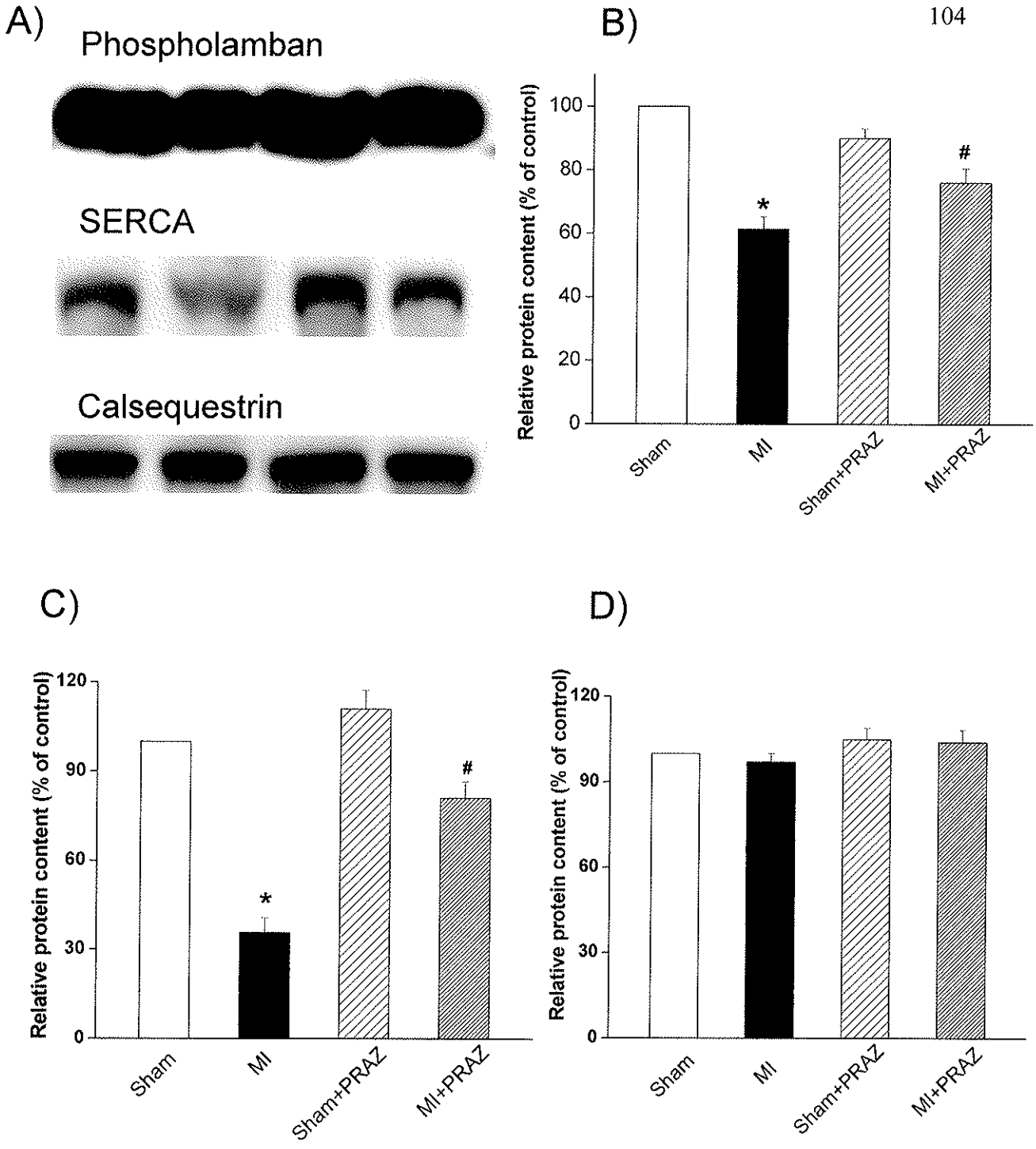


Figure 17. Typical Western blots (panel A) and relative protein content of sarcoplasmic reticular (SR) phospholamban (PLB, panel B), sarcoplasmic reticulum Ca^{2+}-pump ATPase (SERCA2a, panel C), and calsequestrin (CQS, panel D) from sham and infarcted (MI) rat hearts with or without treatment. PRAZ - Prazosin; *$p < 0.05$ compared with sham. #$p < 0.05$ compared with MI group.

Table 26. **Plasma catecholamine levels of rats with and without prazosin treatment at 20 weeks post coronary artery occlusion.**

Parameter	Sham	MI	Sham + PRAZ	MI + PRAZ
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 5 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.

V. DISCUSSION

1. Cardiac Remodeling and Myocardial Performance in the Failing Heart

It has been well established that cardiac remodeling, as depicted by alterations in the size, shape and mass of the myocardium, accounts for the development of CHF, even though the specific mechanisms remain poory understood. It has also been hypothesized that cardiac remodeling occurs as a consequence of increased workload, elevated hemodynamic overload and raised tension on the ventricular wall (4,8-10). In addition, various other aspects including increased levels of hormones that produce vasoconstriction such as catecholamines, angiotensin and endothelins have been suggested to participate in the development of cardiac remodeling (306-309). The hemodynamic investigation showed impaired cardiac function in the MI rats; cardiac dysfunction was evident from decreases in both +dP/d*t* and –dP/d*t* as well as LVSP with an associated increase in LVEDP. Echocardiographic examination of the MI animals also revealed evidence of contractile dysfunction because of the depression observed in parameters such as EF, FS, and CO. General characteristics of the 20 wks MI rats showed significant cardiac hypertrophy because heart wt as well as the heart/body wt ratio were increased. In view of the presence of pulmonary congestion as reflected by an elevated lung wet/dry wt ratio, it is evident that the MI animals were in CHF. Similar changes in CHF rat characteristics have been described by other investigators at different time points of inducing MI (108,112,139,140,290). Furthermore, increases in the dimension of the ventricular chamber (LVIDd and LVIDs), and the posterior chamber wall thickness (LVPWd and LVPWs) as well as a decrease in intraventricular septal thickness (IVSs) suggest that remodeling of the MI heart had occurred in the form of a dilated pumping

chamber and hypertrophied ventricular walls. Dilation of the ventricular chambers and wall hypertrophy are both classic features of HF, which have been observed in our studies of coronary artery ligated rat model of MI. Increased wall stress in developing HF is due to LV dilatation and high systolic pressures needed to overcome excessive afterload. However, enhanced stiffness of the hypertrophied wall takes place at an expense of higher than normal diastolic ventricular pressures, which are essentially transmitted to the left atrium and the pulmonary vasculature. The enhanced cardiac workload related to the onset of an MI generates a compensatory hyertrophic reaction in the viable areas of the heart (80,112,139,140,310); this further leads to progressive dilation and ventricular dysfunction for which the cellular and molecular mechanisms need to be more thoroughly investigated (260). Our results showing a decrease in cardiac function was in agreement with previous publications indicating reduced levels of EF (230,231,256,311,312), FS (80,256,260,310,313,314), CO (260,310), in addition to alterations in myocardial structure and hemodynamics with a decrease in the value for IVS (80,310) and elevated values of LVID (230,256,260,310) and LVEDP (80,256,310,311,313,315).

The finding that treatment of CHF animals due to MI with metoprolol, losartan and prazosin attenuated LV dilatation is in accordance with clinical studies using a wide variety of drugs for the therapy of CHF. There is considerable support that β-adrenergic antagonsim not only attenuates, but also reverses cardiac remodeling in terms of LV dilatation, pressure and hypertrophy (228-230,311,313,316). While the treatment with losartan, metoprolol and prazosin was initiated in rat hearts with healed MI scars, there was no apparent difference in the scar wt or the scar wt/LV wt ratio amongst the treated

and untreated rats. Thus, the effects of these drug treatments on cardiac performance in the MI animals are related to the attenuation of cardiac remodeling in CHF. This view is further attested by our observations that these drug treatments did not affect MAP or heart rate in animals with CHF. Since drug treatments for 8 wks were carried out in 12 wks infarcted animals with impaired cardiac function and cardiac remodeling, this study has provided evidence that these changes were partially reversible with pharmacological interventions such as metoprolol, a well known β_1-AR blocker. Our study also incorporated the effects of losartan (AT_1R blockade) on the cardiac profile, and demonstrated that losartan showed beneficial results in MI animals with CHF that were in harmony with other reports showing prevention of changes in LV dimensions, EF, FS, CO as well as hemodynamical patterns for LVEDP, LVSP, +dP/d*t* and -dP/d*t* due to MI (256,260,262,263,317-319). In relation to the beneficial effects of prazosin, our study revealed new information on α-adrenergic blockade, as there was not a significant amount of literature available on this specific topic. Nonetheless, our observations with prazosin supported the view that attenuation in cardiac remodeling with α_1-blockade are associated with improvements in hemodynamical function and hypertrophic features of heart as well as lung congestion.

On the basis of observations with changes in plasma catecholamine levels, it is well established that high levels of norepinephrine, epinephrine, and dopamine exert toxic biological effects on the heart and that elevated amounts of these plasma catecholamines have an inverse correlation with overall survival rates in CHF (320). The plasma catecholamine levels in CHF are elevated in response to the stimulation of the SNS and consequent release of norepinephrine from the nerve endings, as well as the

release of epinephrine from the adrenal medulla (321-323). Due to the fact that increased plasma levels of catecholamines have been recognized to contribute to cardiac remodelling, contractile dysfunction and associated arrhythmias (321-323), it is possible that these particular hormones play a key role in the process of CHF due to MI. Some investigators have shown that both β-blockade and α-blockade decrease the level of circulation plasma catecholamines and protect the heart from further downstream activation in the remodelling process after myocardial ischemic insult (231,232). On the other hand, certain studies have shown that β-AR antagonists can produce both advantageous and deleterious effects upon treatment in CHF (324,325). Interestingly, these controversial results can be partially explained as to the time period in which drugs are given for treatment (ie in the early or late stages of CHF) (326,327). Since the plasma levels of epinephrine, unlike norepinephrine, were not decreased by β-AR blockade, it is likely that the discrepancy of results for β-adrenergic antagonists may be a consequence of the distinct types of receptors situated at the different locations of action of the sympathetic nerve endings for norepinephrine and the adrenal medulla for epinephrine. Unfortunately, there is not a satisfactory level of understanding of angiotensin blockade and its effects on circulation plasma catecholamines in the rat heart at this time.

2. Ca^{2+}-cycling Activities of SR in CHF due to MI

Even as cardiac remodeling is believed to be involved in the pathophysiology of CHF (4,328,329), there exists a limited amount of literature to support the notion that subcellular remodeling is an intricate process in the advancement of heart failure. The research to date however, has shown that the molecular and biochemical arrangements of

the various subcellular organelles, in addition to their unique architecture and functions, are indeed modified during the development of this disease process (8,129,291,330-334). Particularly, it has been recognized that varying degrees of changes are present amongst the extracellular matrix, the outer SL membrane, the MF, the SR, the MIT and the nucleus, which are in turn dependent upon the nature and stage of CHF (8,129,335). Given that cardiac function is established by meticulously synchronized and closely monitored activities of all the intracellular cardiac organelles as a unit, it can be thought that remodeling of a one or more of these intracellular members can potentially produce a dysfunctioning myocardium during the process leading to heart failure. Even though each individual organelle is responsible for performing a multitude of functions, irregularities in some of the most important actions of these organelles are thought to be primarily implicated in the origins of cardiac dysfunction. Specifically, remodelling of the nucleus and the extracellular matrix has been associated with changes in the gene expression and cellular architecture, respectively (8,58,334,336,337). SL remodeling would greatly disrupt cation homeostasis in tandem with signal transduction by interfering with the finely tuned receptors, cation transporters and cation channels located on this membrane; whereby a disturbance in the MIT power house of the cell would alter the energy production in combination with the redox status that are both intricately linked to the electron transport system and the process of oxidative phosphorylation (58,334,338,339). Moreover, it is the remodeling of the SR that has shown defects in the Ca^{2+}uptake and release functions involved in cardiac contraction and relaxation, which are due to abnormalities in the Ca^{2+}-cycling proteins, whereas remodeling of the myofibrils has been reported to give rise to alterations in this same process of inappropriate cardiac

contractility, together with defects in regulatory proteins (58,334,340,341).

The most important element in the determination of cardiac contractility, the concentration of Ca^{2+}, is attuned by the influx from and efflux to the outside of the cell, as well as by the level of SR function activity throughout contraction-relaxation cycle. Any alterations in this delicate process lead to a disruption in Ca^{2+} homeostasis and cardiac contractile dysfunction. In agreement with previous reports, this study has shown that the Ca^{2+}-uptake activity of SERCA2a is depressed in rat hearts undergoing coronary artery ligation (77,108,112,120,292). It has been suggested that the decline in function could be well traced back to the early genetic processing of SERCA2a, and hence, our study further focused on the SERCA2a mRNA levels through Northern Blot analysis to show a relevant reduction in expression, which has been further acknowledged in other studies (77,81,82,104,108-111,117,120). In view of this finding, we coupled this study with that of protein expression of SERCA2a to find a parallel decrease in its expression, which is also in accordance with other published literature (77,108,109,116,120). Although some studies do not demonstrate a difference in mRNA and protein levels of SERCA2a (114,118), we believe that variations in results may be attributed to mRNA processing and its translation, post translational modification, and alterations in the rates of protein synthesis and degradation.

It is a well known fact that SERCA2a is negatively regulated by PLB, and the existing intimate interaction between the two proteins is the determinant feature that controls the Ca^{2+} content of the SR. Accordingly, we investigated the molecular profile for PLB and found a similar trend as that seen for SERCA2a, namely a decrease in the mRNA and protein expression levels. However, these findings were at odds when

compared to some studies of infarcted rat hearts experiencing CHF (114,293), but were in agreement with other publications (108,111,112). The observed reduction in SR PLB and SERCA2a protein levels were of precise character since the expression of the SR CQS protein remained unchanged in the infarcted failing rat heart. Furthermore, it is the specific balance in the PLB/SERCA2a ratio that ultimately determines SR Ca^{2+}-uptake activity, and the changes observed in this study in relation to overall protein content support this theory of SR dysfunction in Ca^{2+} cycling. In order to restore proper contractility in the failing heart, it is imperative to regain the appropriate PLB/SERCA2a ratio by either decreasing the levels of PLB or conversely increasing the expression levels of SERCA2a. In this study, treatment of infarcted rats with AT_1R blockade, as well as β-AR and α-AR blockade, was found to partially reverse cardiac remodeling at the molecular levels in the SR components such as PLB and SERCA2a. These beneficial effects of losartan (77,108,112,259), metoprolol (228,229) or prazosin are in agreement with those reported with other therapeutic findings. Although the precise mechanisms for alterations in cardiac gene expression and subcellular protein contents are not clear at the moment, increased RAS and SNS activities in combination with further alterations in signal transduction mechanisms in the failing heart have been suggested to play a vital role in the genesis of these molecular changes.

3. Myofibrillar Remodeling in CHF due to MI

Consistent with our previous findings, this study demonstrated a decreased expression of α-MHC mRNA with an associated increased expression of the β-MHC mRNA levels (108,139,140), suggesting a possible fetal gene re-expression like

indoctrination followed by MF remodeling of the infarcted rat hearts. This present study also revealed a depression in the MF Ca^{2+}-stimulated ATPase activity, which in turn gave rise to a decreased level of cardiac function due to the fact that the magnitude of cardiac contractility is directly proportional to the activity of the MF Ca^{2+}-stimulated ATPase (342). The myocardial α-MHC and β-MHC genes have been previously shown to alter their expressional configurations when confronted with a pathological stimulus, whereby changes in the transcriptional control are key determinants in achieving alterations in mRNA production. (343,344). Furthermore, the results that show a reduction in myocardial function in addition of a shift in MF isozymes are in accordance with work previously reported on this specific animal model (345). These particular MF changes have been proposed to explain to some extent the deteriorations in the cardiac contractile cycle of the failing heart. This hypothesis is further strengthened through our findings that cardiac performance is enhanced in the infarcted heart after the attenuation of subcellular remodeling of the MF upon treatment with losartan, metoprolol or prazosin. Considering the critical role of both α-MHC and β-MHC isozymes in deciding the velocity of contraction in myocardial functioning, it is possible that the changes in the expression levels of both isozyme counterparts will heavily influence the MF Ca^{2+}-stimulated ATPase activity (346). Particularly, it has been presented that elevated gene levels of β-MHC mRNA have in fact resulted from increased transcriptional activity, whereas, the changes in the α-MHC mRNA was a direct effect of unusal transcription activity that can be attributed to alterations in post-transcriptional factors, RNA-processing, mRNA stability and the overall turnover rate (344). Additional thoughts on this topic have included possible polyadenylation of the α-MHC, as well as the

inclusion/exclusion of a codon sequencing segment at a glutamine position of in regards to alternate splicing that occurs in the production process (343).

Treatment with losartan and blockade of the RAS was observed to partially prevent the changes noted in the α-MHC and β-MHC isoform mRNA expression, thereby correcting this MF shift that is characteristic in the failing heart. However, exposure to metoprolol and prazosin in blockade of the SNS did not show the same changes after the 8 wks therapy period. These drugs essentially had no significant effect on reversing MF remodeling at the molecular level. Both metoprolol and prazosin, unlike losartan, drug treatments also did not show any success in attenuating the activity of the MFCa^{2+}-stimulated ATPase. Pharmacological intervention with losartan is consistent with beneficial effects reported from previous studies (139), however, there currently is not enough literature available to correlate our negative findings of metoprolol and prazosin treatment on MF remodeling in CHF due to MI. Since the results of our study are in agreement with those of other AT_1R antagonists, candesartan and irbesartan with regards to MF changes (81,257,238). We believe that the beneficial effects on reversing cardiac subcellular remodeling are primarily from the blockade of the RAS and not from the SNS, as was failed to demonstrate with metoprolol and prazosin.

VI. SUMMARY AND CONCLUSIONS

This study was designed to assess the hypothesis that blockade of the SNS with metoprolol and prazosin or RAS with losartan, would provide beneficial effects on cardiac contractile performance through attenuation of cardiac subcellular remodeling in CHF due to MI. Particularly, reversal in changes in the activity of subcellular organelles such as SR and MF would be observed at the gene expression levels for SR PLB and SERCA2a, as well as MF α-MHC and β-MHC. It was also the intention of this study to investigate if the reversal of cardiac and subcellular remodeling was associated with improvement in cardiac performance and hemodynamic profiling upon treatment of CHF animals with pharmacological intervention. In accordance with the results acquired from this study, the following statements and conclusions can be made:

1. Cardiac hypertrophy, chamber dilation, scar formation and pulmonary congestion indicated cardiac remodeling and CHF in rats at 20 wks post coronary artery ligation.
2. Suboptimal cardiac performance and deficient hemodynamic characteristics of animals with CHF due to MI were associated with elevated plasma levels of catecholamines including norepinephrine, epinephrine and dopamine.
3. Reduced SR Ca^{2+}-uptake activity in the failing heart was related to corresponding changes in mRNA and protein expression levels, in addition to the imbalance in the PLB/SERCA2a ratio at both the transcriptional and translational levels following MI.
4. MF remodeling was evident from the isoformal shift in mRNA levels for α-MHC and β-MHC as well as reduction in the activity of the MF Ca^{2+}-

stimulated ATPase.

5. Treatment of 12 wks MI animals with metoprolol, a selective β_1-adrenergic receptor antagonist, and prazosin, an α_1-adrenergic receptor antagonist for 8 wks induced a partial improvement in cardiac function and cardiac remodeling indicating the role of SNS activation in CHF.
6. Treatment of 12 wks MI animals with losartan, an AT_1R antagonist, for 8 wks produced a partial improvement in cardiac function and cardiac remodeling indicating the role of RAS activation in CHF.
7. Treatment of MI animals with losartan, metoprolol and prazosin partially reversed the reduction in SR Ca^{2+}-uptake as well as SERCA2a and PLB content indicating the role of SR in improving cardiac function due to drug therapy in CHF.
8. The inability of losartan, metoprolol and prazosin to affect the increased LVIDd, elevated levels of plasma level of epinephrine and decreased SR Ca^{2+}-release activity in failing hearts indicating the irreversible nature of failing heart at this stage of CHF due to MI.
9. Therapy of the MI rats with losartan, unlike metoprolol and prazosin, partially reversed reductions in MF Ca^{2+}-stimulated ATPase activity as well as gene expression for α-MHC, SERCA2a and PLB, indicating differences in the mode of drug action for improving cardiac function in CHF.
10. Reductions in the elevated levels of norepinephrine by metoprolol and prazosin as well as of norepinephrine and dopamine by losartan and metoprolol indicate that these drugs may produce beneficial actions in CHF

by affecting the SNS in addition to their effects on the α-AR, β_1AR or AT_1R sites in the failing heart.

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