MODULATION OF CARDIAC REMODELING BY ANTIPLATELET AGENTS IN CONGESTIVE HEART FAILURE

BY

SANTOSH K. SANGANALMATH

A Dissertation Submitted to

The Faculty of Graduate Studies

In Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

Institute of Cardiovascular Sciences
Department of Physiology, Faculty of Medicine
St. Boniface General Hospital Research Centre
University of Manitoba
Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION

MODULATION OF CARDIAC REMODELING BY ANTIPLATELET AGENTS IN CONGESTIVE HEART FAILURE

BY

SANTOSH K. SANGANALMATH

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirement of the degree

Of

MASTER OF SCIENCE

ACKNOWLEDGEMENTS

Firstly, I wish to express my gratitude to my supervisor, Dr. Naranjan S. Dhalla for all the guidance, help and encouragement to learn and build my career. To summarize his qualities in one line: he has a sense of purpose; has expectations of success for all students and can demonstrate a willingness to adapt and change to meet student needs. More than scientific knowledge, he gave me the direction for my career. I will always need his blessings throughout my life. I am very much honored to have had him as my supervisor.

I would like to express my warmest thanks to my committee members Dr. Paramjit S. Tappia, Dr. Shetuan Zhang and Dr. Thomas Netticadan, for their advice during my MSc studies. I also wish to thank Dr. Vijayan Elimban, Donald Chapman and Ken Dhalla for their help in my experiments and for creating a harmonious and friendly working environment. I am profoundly grateful to my colleagues Judit Barta, Andrea P. Babick, and Harjot Saini who helped me in my projects. I truly appreciate the efforts of Nancy Gordon and Nicole Whyte for the excellent surgical anesthesia for my animal models.

I owe special thanks to Ms. Susan Zettler, Ms. Eva Little, and Ms. Florence Willerton and all members of Institute of Cardiovascular Sciences for their help and co-operation. I want to express my gratitude to my parents Dr. Prabhulinga Swamy and Jayashree and my brother and sister and my grandparents, who despite being so far away were always there for me with all their love and unconditional support in my endeavors.

ABSTRACT

Cardiac remodeling due to myocardial infarction (MI) is associated with impaired ventricular function that can progress to congestive heart failure (CHF) and has important implications for survival. In spite of several pharmacological and surgical interventions, many patients continue to experience a progressive decline in cardiac function. Therefore, improved methods to attenuate ventricular remodeling and function in ischemic heart failure are needed.

Although, antiplatelet agents are widely used in CHF to prevent platelet aggregation and risk of thromboembolism, the effects of these agents in reversing structural and subcellular remodeling in CHF are not known. Sarpogrelate (SAR), a 5-Hydroxy tryptamine $(5HT)_{2A}$ receptor antagonist and cilostazol (CIL), a phosphodiesterase-III (PDEIII) inhibitor are some of the antiplatelet agents used clinically to prevent restenosis of coronary vessels after stent implantation as well as to prevent platelet aggregation in peripheral vascular disease. We sought to examine if these agents could also prevent structural remodeling in CHF because it would be beneficial for the patients to use a single drug that can prevent platelet aggregation and cardiac remodeling in CHF. The aim of this study was therefore to examine the effects of antiplatelet agents, SAR and CIL in infarcted rats on LV function, myofibrillar Ca^{2+} stimulated ATPase activity, myosin heavy chain (MHC) protein content and gene expression as well as SR Ca^{2+} uptake and release, SR protein content and changes in SR gene expression.

From day 21 post-MI (coronary artery ligation), rats received either SAR (5 mg/day, n=34) or CIL (5mg/day, n=36) for 5 weeks. *In vivo* hemodynamic, echocardiographic and electrocardiographic measurements were made at the end of 8 weeks after coronary

ligation. Sham-operated rats served as controls. At 8 weeks after coronary ligation, untreated MI rats showed marked increase in right ventricular weight (RVW), left ventricular (LV) end-diastolic pressure (LVEDP), LV intrinsic diameters, LV end-diastolic and end-systolic volumes and reduction in rates of pressure development and decay (± dP/dt), LV systolic pressure (SP), mean arterial pressure (MAP), cardiac output, LV stroke volume (SV), LV ejection fraction (EF) and LV fractional shortening (FS). Untreated MI rats also showed signs of CHF as evidenced by an increase in lung wet/dry weight (wt) ratio and development of pulmonary edema. ECGs of untreated MI rats showed marked deviation in ST-segment, premature ventricular complexes (PVCs), loss of R waves, prolongation of QRS duration and prolongation of QT_c (corrected QT) interval. The RVW, LVEDP, LV intrinsic diameters, LV volumes and lung wet/dry wt ratio were reduced, whereas the ± dP/dt, LVSP, cardiac output, LVSV, LVEF and LVFS and MAP were increased towards sham levels in SAR-treated and CIL-treated groups compared with untreated MI. SAR-treated rats also showed preservation of R waves and decrease in QT_c interval. ECGs showed increased incidence of PVCs and ventricular tachycardia (VT) in CIL-treated group. Total mortality during 5 weeks treatment period averaged 27% in untreated MI rats, 11% in SAR-treated rats and 38% in CIL-treated rats. Thus, treatment with SAR significantly reduced mortality whereas treatment with CIL increased mortality.

In order to examine the effects of these antiplatelet agents on subcellular remodeling of myofibrils and sarcoplasmic reticulum (SR), myofibrillar ATPase activity, myosin heavy chain (MHC) isoform expression, α-MHC and β-MHC gene expression as well as SR Ca^{2+} uptake, Ca^{2+} release, SR protein content and gene expression for SR Ca^{2+} pump

ATPase (SERCA2a), ryanodine receptor (RyR) and phospholamban (PLB) were studied in failing hearts following MI. The infarcted animals exhibited a decrease in LV myofibrillar Ca^{2+}-stimulated ATPase activity. MHC-α isoform content as well as MHC-α mRNA levels were also significantly decreased whereas those of MHC-β isoform content and MHC-β mRNA levels were markedly increased. Both SAR and CIL significantly improved the MI-induced changes in myofibrillar Ca^{2+}-stimulated ATPase activity, MHC-α and MHC-β protein content and gene expression. SR Ca^{2+} uptake and Ca^{2+} release was depressed in the failing hearts; protein content and mRNA levels for SERCA2a, RyR and PLB was also decreased. Treatment with SAR and CIL of infarcted animals attenuated alterations in SR Ca^{2+} pump and Ca^{2+} release activities. Changes in protein content and mRNA levels for SERCA2a, RyR and PLB were also prevented by SAR and CIL treatment. The results suggest that while SAR and CIL had beneficial effects on LV function and subcellular remodeling in CHF post-MI, CIL increased mortality, which may be attributed to VT. These findings may have important therapeutic implications of SAR in post-MI CHF.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

5-hydroxy tryptamine (5HT)

Ang-II type 1 receptors (AT1R)

Angiotensin converting enzyme (ACE)

Angiotensin II (Ang-II)

Angiotensin-1 receptor blockers (ARBs)

Arginine vasopressin (AVP)

Atrial natriuretic peptides (ANP)

Brain natriuretic peptides (BNP)

Cardiac output (CO)

Cardiac Resynchronization Therapy (CRT)

Cilostazol (CIL)

Congestive heart failure (CHF)

Coronary artery bypass graft (CABG)

CREST (Cilostazol for RESTenosis)

Cyclic adenosine mono phosphate (cAMP)

Diethyl pyrocarbonate (DEPC)

Ejection fraction (EF)

Electrocardiography (ECG)

Endothelins (ET)

Excitation-contraction (EC)

Extracellular matrix (ECM)

Fractional shortening (FS)

Gi (inhibitory)

Gs (stimulatory)

Hydroxymethyglutaryl coenzyme A (HMG CoA)

Interleukin (IL)

Left ventricular (LV)

Left ventricular assist device (LVAD)

Left ventricular end-diastolic pressure (LVEDP)

Matrix metalloproteinases (MMPs)

Mean arterial pressure (MAP)

Mitogen activated protein kinases (MAPK)

Mitral regurgitation (MR)

Myocardial infarction (MI)

Myosin heavy chain (MHC)

Neutral endopeptidase (NEP)

Nitric oxide (NO)

Norepinephrine (NE)

Partial left ventriculectomy (PLV)

Percutaneous transluminal coronary angioplasty (PTCA)

Phosphodiesterase-III (PDE-III)

Phospholamban (PLB)

Platelet-derived growth factor (PDGF)

Premature ventricular complexes (PVCs)

Protein kinase A (PKA)

Protein kinase C (PKC)

Rate of pressure decay (-dP/dt)

Rate of pressure development (+dP/dt)

Reactive oxygen species (ROS)

Renin-angiotensin-aldosterone system (RAAS)

Ryanodine receptor (RyR)

Sarcolemma (SL)

Sarcoplasmic reticulum (SR)

Sarpogrelate (SAR)

SR Ca^{2+} pump ATPase (SERCA2a)

Stroke volume (SV)

Sympathetic nervous system (SNS)

Tissue inhibitors of MMPs (TIMPs)

Transforming growth factor-β (TGF-β)

Triphenyl-tetrazolium chloride (TTC)

Tumor necrotic factor (TNF-α)

Vascular smooth muscle (VSM)

Ventricular tachycardia (VT)

I. LITERATURE REVIEW

A. Introduction

Congestive heart failure (CHF), an important public health problem, has affected approximately 2.5 to 3 million Americans, while about 750,000 new cases are diagnosed every year in the United States of America (1,2). The economic burden in treating CHF has been estimated to be approximately 5.5 billion dollars, which is 6.5% of the total health care budget; this estimate is more than double the costs of cancer care treatment (3). Since left ventricular (LV) remodeling (changes in cardiac size and shape), due to myocardial infarction (MI) plays a key role in the progression to CHF (4,5), elucidating therapeutic strategies to attenuate the process of cardiac remodeling has become an important area of research in the field of HF (6). Ventricular remodeling in CHF secondary to MI has been shown to occur in 2 phases: an early phase which occurs within 72 hr, and a late phase which occurs within days to months after MI. Infarct expansion, which represents the early phase, may result in ventricular rupture or aneurysm formation. On the other hand, global LV dilation with distortion of ventricular shape and cardiac hypertrophy occurs during the late phase. Failure to attenuate the increased wall stress due to MI is considered to result in progressive LV hypertrophy and impairment in myocardial contractile function.

Although various cardiovascular diseases including coronary artery disease, valvular heart disease, cardiomyopathy, septal defects, hypertension and pericardial disease have also been demonstrated to result in cardiac remodeling and HF, MI is known to be the most common cause of CHF.

B. Acute Ventricular Remodeling

Acute coronary occlusion leads to irreversible cell necrosis, which may be affected, by a number of factors such as preconditioning stimulus (7), collateral flow (8) and the volume of ischemic myocardium. Reperfusion of the ischemic myocardium within 2 - 4 hours of the coronary occlusion when cardiomyocytes are still viable, prevents apoptosis, reduces infarct size and decreases mortality in humans (9). Following one or more MI episodes, the LV undergoes complex changes referred as post-infarction ventricular remodeling (10).

During initial stages of cardiac remodeling after MI, dilation of the LV occurs, which in turn causes acute distension of the noninfarcted myocardium as a compensatory mechanism to restore the stroke volume (SV) and cardiac output (CO). However, in chronic CHF where myocardial damage is extensive, the LV remodeling is inadequate, results in further LV dilation and is thus associated with progressive systolic and diastolic dysfunction. LV volume is considered one of the most important markers of LV dysfunction and a powerful predictor of mortality after MI (11).

Acute myocardial injury leads to a diminished systolic performance and decreased SV followed by a series of histolopathological and structural changes in myocardium (12). This was first demonstrated by Pfeffer and Braunwald (13), who used a post-MI rat model of CHF, and showed that rats with smaller MI had lesser degree of LV remodeling and mortality when compared with rats with larger MI. In early stages of transmural MI, the infarcted myocardium expands with an acute increase in surface area; this process occurs because of thinning and slippage of necrotic myofibrils (14,15,16). Infarct expansion occurs due to early distortion of the inter-myocyte collagen struts, which

results in thinning, dilation, and increased wall stress of the LV (17). Increased wall stress activates several neurohormonal pathways like renin-angiotensin-aldosterone system (RAAS), sympathetic nervous system (SNS), endothelins (ET) and natriuretic peptides-atrial natriuretic peptides (ANP) and brain natriuretic peptides (BNP) that induce compensatory cardiac hypertrophy in the noninfarcted myocardium (18,19,20) and preserve the SV (21). This type of cardiac hypertrophy involves inappropriate increase in length/width ratio of sarcomeres (22). Microscopically, myocyte hypertrophy shows 70% increase in cell volume while mural hypertrophy shows 'in series' sarcomeric replication without any alteration in the sarcomeric length (23). Sympathetic over activity results in augmented shortening and an increase in heart rate that, in turn, results in hyperkinetic movements in the noninfarcted myocardium and temporary circulatory compensation. Natriuretic peptides decrease LV volume and systemic vascular resistance normalizes the filling pressure and improves cardiac function (6).

C. Global Ventricular Dilation and Remodeling in Heart Failure

As previously mentioned, initially there is a progressive increase in LV volume as a result of infarct expansion (24) but subsequently as a result of the increase in wall stress on the residual myocardium, enlargement of noninfarcted myocardium occurs culminating in eccentric hypertrophy of the LV (19,25). In eccentric hypertrophy, there is elongation of cardiomyocytes without increase in cell thickness; this type of cardiac hypertrophy occurs due to the serial deposition of new sarcoplasmic elements (10). According to Law of Laplace, wall tension gradually increases due to eccentric hypertrophy, which in turn leads to global dilation and thinning of the LV wall (26).

Because of this dilation, LV shape eventually changes to spherical from elliptical (27,28) and a functional mitral regurgitation (MR) may result (29). Furthermore, cardiomyocytes may show contractile dysfunction, which is further blended by the increased LV mass/LV volume ratio, leading to myocardial ischemia because the blood supply and oxygen demand cannot cope with increased demands of the hypertrophied LV (10).

Extracellular Matrix (ECM) plays a significant role in cardiac remodeling, infarct expansion and scar formation after MI (30). About two-third of heart cells are composed of cardiac fibroblasts, which synthesize and secrete type-I collagen; the main function of cardiac fibroblasts is to regulate ECM levels by synthesis and deposition of matrix molecules, as well as matrix breakdown and turnover by matrix metalloproteinases (MMPs) and by maintaining mechanical tension on the collagen strut (31). Type-I collagen has the tensile strength of steel and represents 90% of the total collagen content (32). Type-I collagen forms a structural framework, which maintains myocyte alignment during contraction and relaxation (32). Procollagen type III N-peptide is a significant marker of myocardial fibrosis and it is shown to be significantly elevated in LV hypertrophy and HF (33).

In the early phase of infarct expansion after MI, edema and inflammation occur at the region of ischemia, which are followed by scarring of myocardium over a period of weeks to months. The infarcted myocardium can thin and elongate before the tensile strength is restored (34). Thinning of the infarcted myocardium occurs due the "slippage" between muscle bundles, which in turn results in a decrease in the number of myocytes (35). In the collagen matrix, myocytes are held in an organized fashion by the intercellular struts, which is broken following an ischemic attack (36). Thus, ischemic

zone shows diminished force generation followed by decreased contractile performance due to uncoupling of these cardiac cells. Moreover, there is a diminished blood supply and oxygen to the infarcted area because of the interruption in collagen fibres, which bridges myocytes to the capillaries (37). During the process of healing, the connective tissue provides resistance against further myocardial stretch by connecting disrupted myocyte fibres (38). After days to weeks of MI, granulation of tissue is followed by replacement of necrotic tissue with scar formation interwoven with muscle fibres (39). Clinically, infarct expansion is reflected by a decrease in SV, CO, and ejection fraction (EF) (40), which can be evaluated by echocardiography, showing aberration of the ventricle, as well as thinning and elongation of the noncontractile infarcted myocardium (41). These patients are more likely to experience complications such as CHF, aneurysm and cardiac rupture (41-44). Furthermore, Pirolo et al (45) have shown that a larger degree of infarct expansion is associated with endocardial thrombus and endocardial fibroelastosis.

***a. Role of MMPs and TIMPs in cardiac remodeling*:** Apoptosis has been observed in both experimental models and in myocardium from patients with HF due to MI (46,47). Apoptosis is also observed in cultured cardiac cells which are stimulated by a number of factors like norepinephrine (NE), angiotensin II (Ang-II), cytokines, mechanical stretch and pressure overload (48-50). Following an ischemic attack, myocyte necrosis is associated with edema and inflammation followed by granulocyte infiltration and release of proteolytic enzymes. Role of MMPs, a family of zinc-dependent collagenases and tissue inhibitors of MMPs (TIMPs) in cardiac remodeling, collagen degradation and infarct expansion have gained fresh impetus lately (51). Serum levels of

MMP-1 are found be elevated during the first 14 days following MI, during which period collagen breakdown predominates. Following this, serum TIMP-1 levels shift from lysis to fibroblast infiltration, collagen deposition and scar formation (52). MMPs are activated by several factors like cytokines, reactive oxygen species (ROS), Ang-II and ET (53,54).

Several MMPs are shown to degrade the components of ECM (33). Clinically, increased levels of MMP-2, MMP-3, MMP-9 and MMP-13 have been reported in myocardium of patients with CHF. Several experimental studies using transgenic and knockout mice models have also shown the role of MMPs and TIMPs in cardiac remodeling and LV dysfunction. Kim et al (55) showed loss of cardiac interstitial collagen and cardiac dysfunction in mice overexpressing MMP-1. In contrast, Ducharme at al (56) showed attenuation of LV hypertrophy and decreased collagen accretion by targeted deletion of MMP-9 in infarcted region after MI. Following MI, TIMP-1 has been shown to impair scar formation and revascularisation and prevent cardiac rupture. Roten et al (57) showed LV hypertrophy and reduction in collagen in TIMP-1 deficient mice. In a model of pacing induced HF, MMP inhibition early in the remodeling process was found to decrease the LV dimensions and improve cardiac performance (58). Thus, pharmacological inhibition of MMPs can be targeted as a potential mode of treatment for ventricular remodeling and HF (59).

b. Role of cytokines and tissue growth factors in cardiac remodeling: Inflammatory cytokines are produced in different cellular systems of the body including myocytes, endothelial cells and macrophages. Cytokines like tumor necrotic factor (TNF-α) and interleukin (IL)-6 play a key role in cardiac remodeling. Clinical studies have shown that levels of plasma cytokines like TNF-α, IL-6 and IL-1β are elevated in patients

with advanced CHF (60). Experimental studies have also shown that an increase in TNF-α promotes LV remodeling and cardiac dysfunction (61). The pioneering study conducted by Torre-Amione and colleagues (62) reported increased levels of TNF-α in patients with symptomatic LV dysfunction. Moreover, myocardial TNF-α has also been shown to be elevated in failing hearts (63-65). Clinical trials have revealed persistent TNF-α mRNA and protein levels in cardiomyopathic patients (63).

Bozkurt and colleagues (66) have also made several observations upon infusing TNF-α in rats with CHF and found a decrease in LV fractional shortening (FS) after 5 days which effect was reversible upon the removal of TNF-α infusion. Furthermore, a time-dependent increase in LV end-diastolic dimension was observed after TNF-α infusion (66), in addition to a small increase in the average LV mycocyte cross-sectional area and decrease in number of myocytes across the transmural thickness of LV wall (66).

It has been shown that activation of TNF-α receptor increases the production of MMPs, which indirectly aids in the degradation of the ECM components (61,67). By the enhancement of Ang-II effects on cardiac fibroblasts, TNF-α is also considered to modulate cardiac remodeling in MI (33). It is pointed out that cytokines are known to stimulate iNOS (68), which in turn, may lead to the formation of free radicals and development of oxidative stress (69). It is also known that Ang-II promotes the generation of oxygen free radicals (70) whereas catalase, an antioxidant, has been reported to prevent cardiac hypertrophy induced by Ang-II or TNF-α (71). In addition to inducing cell necrosis, cytokines are known to affect Fas receptors, and thereby increase caspase activity and produce apoptosis (72). Stretching of cardiomyocytes has also been

shown to promote Fas expression (73) and TNF-α has been reported to upregulate the expression of Ang- II type 1 receptors (AT1R) in cardiac fibroblasts (74). It should also be noted that growth factors like transforming growth factor-β (TGF-β), fibroblast growth factor, platelet-derived growth factor (PDGF) and Ang-II mediated TGF-β1 production have been shown to play a critical role in regulation of ECM production, cardiac hypertrophy and fibrosis (33). Schultz et al (75) have reported that there were no significant change in LV mass and cardiac function when TGF-β1 deficient mice were subjected to chronic subpressor doses of Ang-II but in contrast, Ang-II treated wild type mice showed cardiac hypertrophy and depressed cardiac function. Van Mawel et al (76) have shown that mechanical stress increases TGF-β1 expression through Ang-II and ET-1 in cardiomyocytes.

c. Noninfarcted myocardium in ventricular remodeling: Although MI induces cardinal changes in the infarcted myocardium, progressive alterations also occur in the noninfarcted segment (77). Theroux et al (78) showed a relative shortening of myocardial fibres and increase in the end-diastolic length of sarcomeres in the noninfarcted segment. This change occurs mainly as a compensatory mechanism to maintain SV and CO because of increased LV end-diastolic volume/muscle mass ratio, an index of overall wall tension (79). Following MI, wall stress and diastolic pressure increase significantly, which are directly proportional to the size of the infarct. Such an increase markedly diminishes the ventricular performance (80) as increased wall stress causes anatomic and cellular changes in the ventricle leading to dilation (80) and eventually to volume overload (81). The cardiac enlargement causes a proportional increase in LV chamber radius but only a slight increase in wall thickness and accommodates the excess blood

volume and returns diastolic pressure to normal. This type of hypertrophy is evidenced at the cellular level by elongation in cell length, which is out of proportion to the increase in diameter (40,81,82). Clinical studies have also shown an enlarged LV cavity even in the absence of elevated filling pressures in patients who have survived an attack of MI (83,84,85). As a result of this global ventricular dilation, the amount of ventricular damage is directly proportional to EF and thus, cardiac function worsens with severe myocardial cell damage (86,87,88). Moreover, EF decreases as end-diastolic volume increases in presence of chamber hypertrophy (40). SV and CO do not decline in acute distension because of the chronotropic and inotropic mechanisms that maintain the cardiac pump (89,83) but with chronic distension, mismatch occurs between volume overload and increased wall stress leading to the development of clinical HF (81,90). These events are associated with the activation of various neurohormonal systems post-MI; the degree and time frame of activation depend on infarct size and magnitude of LV dysfunction (91). These neurohormonal systems include the RAAS, SNS, natriuretic peptides, ETs, cytokines and arginine vasopressin (AVP) (92).

i. Sympathetic Nervous System:

SNS is activated very early after the onset of MI, which has both positive chronotropic and inotropic actions (93) and in fact, the sympathetic drive is increased >1000 fold in HF (94). This SNS overactivity leads to stimulation of β- and α-1 receptors through different signaling pathways, which results in the activation of protein kinase A (PKA) and protein kinase C (PKC). Ultimately, cardiac remodeling and hypertrophy occurs as a result of transcription factor production and re-expression of fetal gene program due to subsequent stimulation of mitogen activated protein kinases (MAPK) and

expression of proto-oncogenes (33). In the acute phase of MI, SNS activation is adaptive and helps to maintain CO and blood pressure. However, sustained activation of this system becomes pathological and contributes to ventricular remodeling and increased cardiac wall stress, which leads to ventricular dilation and ultimately HF (95,96). Chronic SNS activation is likely to deteriorate cardiac function by increased generation of superoxide anions and development of oxidative stress (97-99). On the other hand, nitric oxide (NO) that is present in the myocardium (100) has been shown to prevent many processes associated with cardiac remodeling. There is clinical evidence that chronic treatment with nitrates attenuates cardiac remodeling, post-MI (101). Nitric oxide (NO) has been observed to decrease Ang-II induced cardiomyocyte hypertrophy (102), increase angiogenesis (103) and decrease cardiac fibrosis (104). Recently in a murine model of MI, Scherrer-Crosbie et al (105) have demonstrated that the presence of eNOS improved cardiac function and attenuated remodeling by decreasing myocyte hypertrophy in noninfarcted segment. Endothelial NO is also known to cause reduction in both afterload and preload by vasorelaxation (106).

ii. Renin-Angiotensin-Aldosterone System:

Activation of the RAAS induces systemic vasoconstriction and plays a key role in the pathophysiology and progression of ventricular remodeling after MI (107). RAAS also stimulates other systems including AVP and aldosterone that contribute to maintain adequate intravascular volume (108). AVP decreases excretion of water and electrolytes and increases blood volume (109). Chronic activation of RAAS has been found to be detrimental and contributes to the progression of ventricular remodeling in CHF (110). Renin that is released from the juxtaglomerular cells of the kidney in response to multiple

factors including SNS activity, reduced sodium absorption by distal tubules or AVP release (111), acts on angiotensinogen and converts it to Ang-II. Ang-II acts as a local growth factor and promotes LV hypertrophy following MI (107). Ang-II has several physiologic functions that are important in fluid regulation (112), vasoconstriction and stimulation of aldosterone from adrenal cortex. Aldosterone in turn aids in sodium ion resorption by distal tubles. The activity of RAAS including angiotensin-converting enzyme (ACE) is markedly increased in HF and this produces Ang-II, which results in LV remodeling (113). Ang-II has many adverse effects on cardiac tissue; these include (a) playing a significant role in cardiac hypertrophy by increasing DNA synthesis in both cardiocytes and fibroblasts, (b) increasing coronary permeability and thus allowing the tissue growth factors to diffuse inside the myocardium, resulting in adverse remodeling (114), and (c) that it causes necrosis due to its cytotoxic effects on cardiomyocytes (115).

Although aldosterone is a circulating hormone produced in suprarenal gland, it is now clear that aldosterone and its receptor are present in heart and blood vessels (116). Levels of aldosterone are significantly increased following MI and in CHF (117). In addition to avid fluid retention through its mineralocorticoid effects, aldosterone is also a potent mediator of myocardial fibrosis (118). Aldosterone is known to affect vascular compliance and endothelial function and induce ischemia (119). It is also pointed out that aldosterone has also been shown to cause an increase in SNS activity and electrolyte disturbances by increasing the urine excretion of magnesium and potassium, which in turn lead to myocardial apoptosis and ventricular arrhythmias (33). In addition to aldosterone, AVP, an antidiuretic hormone, is considered to affect the development of CHF. This hormone is synthesized in neurosecretory cells of the paprventricular and

supraoptic nuclei of hypothalamus (120), and has been shown to regulate various body functions including body osmolality, blood volume, cell contraction, blood pressure and adrenocorticotropin secretion (110). The actions (vasoconstriction and cardiac hypertrophy) of AVP are mediated by V1a, whereas water and sodium regulation are mediated by V1b and V2 vasopressin receptor subtypes (121). In patients with CHF, circulating AVP levels are elevated and associated with significant cardiac decompensation and hyponatemia (122,123,124).

iii. Natriuretic Peptides:

Several vasodilating peptides are produced in cardiac tissue that has counteracting effects on cardiac remodeling; some of these include bradykinin, ANP and BNP. Following MI, ANP is released from the right atrium and BNP from the ventricles in response to right atrial stretch and right ventricular (RV) wall tension, respectively; thus, there occurs an increase in systemic levels of these peptides (125). Both ANP and BNP cause a reduction in afterload through a combination of peripheral vasodilation and natriuresis (125). These effects are opposite to that of sympathetic over activity and activated renin-angiotensin axis. Natriuretic peptides exert their beneficial effects on hemodynamics, fluid balance, and renal function initially after MI. In addition to this, natriuretic peptides inhibit cardiac hypertrophy and have beneficial effects on cardiac remodeling (126). Bradykinin is a vasodilator and has anti-remodeling effects by inducing NO formation (33). One of the mechanisms by which angiotensin converting enzyme (ACE) inhibition has anti- remodeling effects is by the increased production of bradykinin. Beneficial effects of bradykinin come from the experimental studies conducted on bradykinin β2-knock-out mouse in which LV chamber size, as well as

perivascular and reparative fibrosis was significantly greater than in wild type mouse.

iv. Endothelins:

ET is released from the vascular endothelium and is a potent vasosconstrictor and hypertrophic peptide, which produces actions by acting upon ETA receptors (93). Following an ischemic attack, the systemic levels of ET are elevated to maintain blood pressure (127) but later, due to its hypertrophic effects, it produces cardiac remodeling and ventricular dilation (128). ET levels are significantly increased in HF (129); experimental rat model of CHF has also shown an increase in ETA and ETB receptor densities (130). ET leads to vascular smooth muscle cell proliferation by producing various factors like PDGF and TGF-β1 in vascular tissue. It is also known to increase thrombsis and ischemia by increased production of plasminogen activator inhibitor-1 (PAI-1). The activation of ETA receptor leads to cardiac hypertrophy through Gqa stimulation (33). ET has also been reported to act through ET1 indirectly and cause pathologic hypertrophy with early gene expression, as well as lead to myocardial apoptosis. Furthermore, ET was observed to synthesize ECM proteins like fibronectin, collagen and laminin and stimulate fibroblast proliferation directly (131) thus resulting in myocardial fibrosis (132). It is also known that ET stimulates the secretion of other neurohormones like NE, Ang-II and aldosterone. ET, by inducing the release of cytokines, stimulates the activity of lipooxygenease and increases the production of monocyte chemoattractant protein-1 for inducing the inflammatory reaction.

D. Subcellular Remodeling in Heart Failure

Subcellular remodeling of the sarcoplasmic reticulum (SR), myofibrils, sarcolemma (SL) and mitochondria has been reported to occur in cardiac hypertrophy and CHF (133,134). Cardiac remodeling during the development of CHF has been reported to be invariably associated with subcellular remodeling, which is reflected by alterations in the molecular structure and biochemical composition of different subcellular organelles in cardiomyocytes.

a. Sarcolemmal and Saroplasmic Reticular Remodeling in CHF:

Numerous structures in myocardial cell are adversely affected by an ischemic insult of which SL and SR changes are more prominent. These two membranes play an important role in excitation-contraction (EC) coupling in the heart. Cardiac depolarization results in small influx of Ca^{2+} via voltage dependent SL channels; SL Na^{+}-Ca^{2+} exchanger also plays some role for Ca^{2+} influx in EC coupling under certain conditions (135,136). Ca^{2+} influx results in a further release of large amount of stored Ca^{2+} from SR via Ca^{2+} release channels. Myocardial contraction results in response to increased cytosolic Ca^{2+} when troponin-tropomyosin complex surrounding myosin allows sliding of thick myosin filaments over thin actin filaments. Relaxation results when there is Ca^{2+} uptake into SR; Na^{+}-Ca^{2+} exchanger and Ca^{2+} pump of SL also extrude Ca^{2+} from the cell into the extracellular space (135,136). In HF, EC coupling is disturbed because of changes that occur in SR and SL. It is now clear that in failing hearts, there is an increase in intracellular Ca^{2+} levels, which causes overload and contractile dysfunction (137,138). Ca^{2+} overload cause damage to contractile apparatus, energy generating systems and membranes which all lead to CHF.

SL maintains contractile function through various cation transporter activities including ATP-dependent Ca^{2+} uptake, Ca^{2+} stimulated ATPase activity, Na^{+}-Ca^{2+} exchanger and Na^{+}-K^{+}-ATPase (135,138). It has been shown that the number of Ca^{2+} channels in SL is decreased in rats with CHF (139). In a clinical study, level of mRNA encoding of Ca^{2+} channels was decreased in HF (140). In cardiomyopathic hamsters with CHF, the Ca^{2+} channel density was unchanged (141). Thus, the Ca^{2+} channel changes in CHF depend on type of HF. SL Na^{+}-K^{+}-ATPase pump and Na^{+}-Ca^{2+} exchange activities are shown to be decreased in MI (142,143). Na^{+}-Ca^{2+} exchange and Ca^{2+} pump activities were extensively studied using genetic cardiomyopathic hamster model of HF. In UM-X7.1 strain of cardiomyopathic hamsters, Na^{+}-Ca^{2+} exchange and Ca^{2+} pump activities were decreased. Na^{+}-Ca^{2+} exchange activity was also found to be decreased in BIO 14.6 cardiomyopathic hamsters (141,144). In a rat model of CHF, it should be noted that there was depressed SL Na^{+}-Ca^{2+} exchange with no changes in Ca^{2+} pump activites in the viable LV (143). Depression in SL Na^{+}-K^{+}-ATPase activity was observed in failing hypoxic rat heart (145), UM-X7.1 cardiomyopathic hamsters (146), rabbits with pressure load hypertrophy (147) and viable LV in rats with CHF (142). However, the Na^{+}-K^{+}-ATPase activity was increased in BIO 14.6 strain of cardiomyopathic hamsters and canine hearts with volume/pressure overload (148,149). Such diverging changes support the hypothesis that SL changes depend on the type of the disease. In addition to SL, marked alterations in SR membrane have been reported to occur in the failing heart. It is pointed out that Ca^{2+} uptake into SR is accomplished by a Ca^{2+} stimulated ATPase pump and release of Ca^{2+} occurs via ryanodine sensitive Ca^{2+} channels (150). Increased Ca^{2+} efflux via ryanodine-sensitive Ca^{2+} channels (151), decreased SR Ca^{2+} stimulated ATPase

activity (152) and depressed mRNA signal for gene expression (153) have been shown to decrease in Ca^{2+} uptake into SR in CHF due to MI.

b. Myofibrillar and Mitochondrial Remodeling in CHF:

Cardiac contractile apparatus contains two important proteins actin and myosin, the interaction of which is modulated by troponin and tropomyosin. Experimental studies with failing hearts have shown a shift in myosin isozyme content from V1 toV3 (154-157). This shift is also known to occur at the transcriptional level and has been demonstrated in hearts subjected to cardiac overload (158). It is believed that alterations in the myosin molecule in the failing heart cause changes in contractile properties and the sensitivity of contractile unit to Ca^{2+}. It needs to be emphasized that during the development of Ca^{2+} overload, enough Ca^{2+} accumulates inside the mitochondria, which results in decreased production of high-energy phosphates and in turn, cellular dysfunction (159). Phosphocreatine is the main high-energy phosphate store in heart and its concentration is known to be decreased in CHF (160). Creatine kinase is an enzyme, which mediates transfer of the phosphoryl group to and from phosphocreatine and is known to switch isoforms in response to myocardial stress (161). In a study on post-MI energy changes conducted by Neubauer et al (162), it was found that ATP levels of noninfarcted tissue were same as that of control animals. However, phosphocreatine levels, creatine content and mitochondrial creatine kinase levels were significantly reduced in infarcted rats; no changes in the glycolytic pathway were observed in this study.

c. β-adrenoceptor Mechanism in Cardiac Remodeling and CHF

Stimulation of SNS in HF leads to excess release of NE, which bind to β-receptors on the SL and the signal, is transmitted through guanine-nucleotide (G) proteins to adenylyl cyclase. G proteins may be Gs (stimulatory) or Gi (inhibitory) that modulate activity of adenylyl cylase and subsequent formation of cAMP. The increase in cAMP activates cAMP-dependent PKA, which mediates phosphorylation of target proteins including L-type Ca^{2+} channels, phospholamban, troponin-I and troponin-C (136). However, the density of β-receptors was decreased without any changes in the affinity for agonists in post-MI CHF (163). Furthermore, Boehm and colleagues (164) studied the β-receptor density in human HF and found that β_1 receptor density was significantly decreased, while β_2 receptor density did not change. G_s activities and densities did not change, while G_i proteins were increased and adenylyl cyclase levels decreased in postinfarcted hearts (163,164). Yamamoto et al (165) studied β-adrenoceptor-G-protein-adenylate cyclase complex in rat hearts with ischemic heart failure and found that G_s and G_i function was decreased but adenylate cyclase, B_{max}, K_d, the amount of G_s and G_i did not change in ischemic heart failure. Therefore, these researchers concluded that a dysfunction in Gs might contribute to the contractile abnormalities in ischemic heart failure. Sethi et al (166) studied the alterations of γ-proteins in CHF in UM-X7.1 cardiomyopathic hamsters and found depressed adenylyl cyclase activation is not only due to increased content and bioactivity of G_i proteins but the functional uncoupling of G_s proteins from adenylyl cyclase enzyme may also be involved in this type of CHF. Since catecholamines are known to modify Ca^{2+} influx via the generation of cAMP through β-adrenoceptor pathway (136), it is likely that the decrease in catecholamines response in

the failing heart may be due to a decrease in Ca^{2+} influx. This results in less Ca^{2+} being released from SR that results in decreased contractile force generation in the failing heart. Because phosphorylation by cAMP dependent protein kinase is also known to increase Ca^{2+} uptake by cardiac SR and augments myocardial relaxation (136), a defect in phosphorylation at different subcellular organelles including SR can be seen to account for diastolic abnormalities in the failing heart.

E. Pharmacological Interventions for Attenuating Ventricular Remodeling

a. ACE Inhibition:

The main mechanism of action of ACE inhibition is believed to be by decreasing the systemic levels of circulating Ang-II and subsequently increasing bradykinin levels, which in turn has anti-remodeling effects. Many investigators have shown that ACE inhibitors decrease the tissue levels of Ang-II by reducing its production and suppressing the cardiac dilation due to increased cardiac workload (107). Moreover, ACE inhibitors decreased the plasma aldosterone levels and prevented sodium retention and fluid accumulation (107). Previous studies have shown that different ACE inhibitors prevented the subcellular changes, which occur in the myocardium at the level of SL, SR, ECM and mitochondria in failing hearts due to MI (107). Also blockade of ACE attenuated the impairment of β-adrenergic signal transduction mechanisms in HF (107). McDonald et al (167) studied the effects of ACE inhibition in animal model of HF induced by transmyocardial direct current shock and was shown to prevent cardiac hypertrophy (167). Clinical studies with ACE inhibitors in patients with LV dysfunction have shown to prevent cardiac dilation (168). Angiotensin-1 receptor blockers (ARBs) were also

observed to have a prominent beneficial effect in preventing ventricular remodeling. Schieffer et al (169) compared the effects of losartan with enalapril in a rat model of CHF and showed that losartan was as beneficial as enalapril in reducing cardiac hypertrophy and myocardial fibrosis. ARBs have also been reported to prevent changes in the subcellular and molecular levels in failing heart (107). It should be noted that interventions other than these producing blockade of RAS have also been observed to exert beneficial effects in CHF. For example, inhibition of AVP activity in patients with symptoms of volume overload including pulmonary edema, congestion with hyponatremia by blocking V2 receptors were observed to reduce congestion and pulmonary edema (169), whereas V1a receptor blockade reduced plasma NE and Ang-II levels (170). These beneficial effects are found without deteriorating renal function or electrolyte abnormalities (169).

b. β-Adrenergic Receptor Blockade:

It is now well understood that long-term activation of the SNS releases excessive amounts of catecholamines, which in turn have deleterious effects on the heart i.e. cardiac dysfunction, death of cardiac myocytes and arrhythmias in CHF (171). β-blockers can attenuate many of these deleterious effects of catecholamines and arrest the structural changes that occur during progression of CHF (172), as well as increase survival in experimental models (173). Long-term administration of β-blockers has been shown to reduce mortality by 20 % in a large number of randomized controlled trials in patients with MI. It is believed that the reduction in mortality by β-blockers is probably by preventing myocardial ischemia and reducing the chances of arrhythmias (174).

The basis of this anti-ischemic effect is due to the reduction of heart rate and

myocardial oxygen demand and that of anti-infarction effect is related to the ability of these drugs to decrease the risk of atherosclerotic plaque rupture. In patients with CHF, β-blockers reduce myocardial oxygen consumption even when the heart rate is maintained at a high constant rate by atrial pacing (175). β-blockers have been shown to be cardioprotective due to anti-arrhythmic effects (173), anti-thrombotic effects (176), decreased myocardial oxygen demand (177) and prevention of atherosclerotic plaque rupture (178). The mechanism of action of β-blockers is still not clear but several different pathways have been suggested like attenuation of toxic effects of catecholamines (172), reversal of pathological remodeling (179), prevention of myocardial cell death (180), up-regulation of β-adrenergic receptors (181) and protection from autoantibodies against β_1-receptors (182). Theoretically, non-selective β-blockers are expected to be more effective in the treatment of CHF than selective agents due to several reasons. Firstly, both β_1- and β_2-receptors mediate the toxic effects of catecholamines on the myocardium, directly (183). Therefore, drugs that block both β_1- and β_2-receptors may be more effective in producing long-term beneficial effects than selective β_1-receptor blockers. Secondly, catecholamines stimulate their own release via pre-synaptic β_2-receptors (183), so non-selective agents can achieve better adrenergic drive control. Thus carvedilol being a non-selective β-blocker is considered more beneficial when compared to other β-blockers in attenuating cardiac remodeling in CHF. Metoprolol, a β_1 selective blocker, was compared with carvedilol in HF in which carvedilol reduced LV volumes significantly but metoprolol failed to show the similar results (184,185). Moreover, only a small number of trials have reported a decrease in cardiac volumes with β_1 selective blocking agents (186).

c. Aldosterone Receptor Antagonists:

Although Ang-II is known to stimulate the release of aldosterone and treatment with ACE inhibitor can block both Ang-II and aldosterone, excess aldosterone production may "escape" through non Ang-II dependent mechanism (187). Thus it is imperative to block the aldosterone receptor as this escape mechanism has several adverse effects on the heart including myocardial fibrosis, sodium retention, loss of potassium and magnesium, increase NE release, cardiac hypertrophy and endothelial dysfunction. With this background, ARBs were developed in the treatment of heart failure. Both spironolactone and eplerenone are widely used in HF and has shown to reduce cardiac fibrosis and decrease LV mass (33). In an animal model of CHF, spironolactone significantly decreased cardiac volumes and circulating procollagen levels resulting in improved cardiac performance and decreasing myocardial fibrosis (188). In a clinical trial, spironolactone was administered in conjunction with other standard medications for HF and significantly prevented the LV remodeling (189). Spironolactone treatment was also associated with a reduction in ventricular premature contractions and non-sustained VT, reduced LV mass (189), reduced LV volumes, increased EF and improved diastolic parameters (189). Since eplerenone is a novel selective competitive antagonist of aldosterone receptor (189), it was found to be more beneficial than spironolactone as it exhibited reduced binding to androgen or progesterone receptors and thus produced less endocrine side effects (189).

d. Neutral Endopeptidase and Cytokine Inhibitors:

Neutral endopeptidase (NEP) inhibitors block the conversion of Ang-I to Ang-II and the catabolism of bradykinin, ANP and BNP (190). Thus by inhibiting both ACE

and NEP, NEP inhibitors increase natriuretic and vasodilatory peptides as well as the half-life of other vasopeptides, bradykinin and adrenomedullin (191). Inhibition of both NEP and ACE is known to be more beneficial for patients in HF than ACE inhibition alone. Omapatrilat is a vasopeptidase inhibitor inhibiting both ACE and NEP activities (190). Clinically, ompatrilat reduced end-diastolic volumes over a period of 12 weeks in absence of ACE inhibitor or Ang-II antagonist therapy (192). However, the patients develop tolerance to this drug rapidly as the peptides have short half-life. Furthermore, other adverse effects like hypotension and bradycardia have limited their use (93). Trippodo et al (193) have shown that cardiomyopathic hamsters treated with omapatrilat had less mortality when compared to captopril treated hamsters.

Etanercept is a soluble recombinant TNF receptor protein, which binds TNF-α and thus inactivates the effects of TNF-α (194). It has been shown by Deswal et al (195) that etanercept-treated patients had improved LVEF when compared to HF patients on placebo. In another clinical trial, different dosages of etanercept in advanced HF administered to patients for a period of 3 months, showed improved LV function (196). It is well known that TNF-α administration leads to progressive LV dilation (33); cytokine production can also be suppressed by treatment with ACE inhibitors and β-blockers.

e. Statins:

In recent years, the use of hydroxymethyglutaryl coenzyme A (HMG CoA) reductase inhibitors has increased in coronary artery disease as these agents may have role beyond cholesterol lowering effects. In an experimental study of rat model of CHF by coronary ligation, statins were shown to improve ventricular function by limiting cardiac fibrosis (197,198). It is believed that blockade of HMG CoA reductase disrupts

the mevalonate pathway (199). These agents are also known to depress the detrimental cellular effects of Ang-II and decrease the expression of AT1 receptors by inhibiting HMG CoA-mevalonate-GGPP pathway (200). It is well known that ROS enhance proliferation of vascular smooth muscle (VSM) cells and induce apoptosis of endothelial cells resulting in cardiac remodeling (201). Statins can reverse this mechanism by down-regulation of AT1 receptor gene expression and thus decreasing Ang-II induced release of ROS (199). Statins are known to have sympatholytic effect by up-regulation of the eNOS expression (202,203). Pliquett et al (204) showed lowered plasma NE levels with simvastatin on normolipedemic rabbits with HF. In patients with advanced CHF, simvastatin was reported to modify the effect of ET-1 by reducing immunoreactive ET-1 (205). Statins were also studied in relation with cytokines and were shown to decrease the levels of TNF-α (25) and IL-6 (19). Havashidani et al (198) studied the effects of fluvastatin after MI and found that the drug decreased the levels of MMP and increased the levels of TIMP-2 resulting in an improvement in cardiac function and reduction in LV dilation and cardiac fibrosis. Furthermore, statins lower LDL-cholesterol and reduces ischemia and its adverse effects (206,207).

f. Endothelin Antagonists:

Several experimental studies have shown the beneficial effects of ET antagonists on cardiac remodeling either by ETA receptor blockade or ETA and ETB receptor blockade in CHF and increase survival (208-211). However, adverse effects of ETA blockade have also been reported by several investigators (129,212). When these agents are used early following MI, they may impair scar healing and lead to ventricular dilation. In contrast, this is not the case with combined ETA and ETB blockade with

bosentan (213). But in chronic stages of MI, both agents affect the remodeling to the similar extent. Sakai and colleagues (214) used BQ-123 in rats with chronic MI and showed significant reduction in cardiac hypertrophy and chamber dilation. Bosentan, an oral nonpeptide and a dual ET receptor antagonist is a novel drug, which is shown to improve ventricular remodeling in HF. Chronic administration of this drug in the MI rat HF model significantly reduced adverse cardiac remodeling, decreased LV filling pressures, LV volumes, cardiac fibrosis and catecholamine levels, and increased CO (215). Bosentan has also shown beneficial effects in acute HF, administered 3 hours and 24 hours after coronary occlusion (216,217). 8 weeks of bosentan treatment in the same model resulted in improved cardiac function (218) and normalized cytokine levels (219). Moreover, bosentan has been shown to decrease mortality in rats with CHF (215). Apart from this model, bosentan also attenuated adverse remodeling in Dahl-sensitive rat model (220) and pacing induced rat models of HF (221).

F. Surgical Interventions in Ventricular Remodeling

Despite so many pharmacological interventions for CHF, 50% patients die within 3 years (222). Although cardiac transplantation remains the ultimate surgical therapy for CHF, it is applicable only to small percentage of patients because of unavailability of donors and thus it is necessary to consider alternative surgical approaches in treating end-stage heart disease. Cardiac transplantation is known to have excellent results with survival rate being 85% at the end of one year and 68.5% at the end of five years (223).

a. Surgical Restoration of the Left Ventricle:

Batista et al (224) were the first to suggest the idea of surgical restoration in

patients with signs and symptoms of CHF with dilated and poorly contracting heart. The main idea of this procedure was to reduce the ventricular volume by partial left ventriculectomy (PLV), by a resection of the lateral free wall of the LV. The rationale of this procedure was to restore wall thickness/wall radius ratio to normal and thereby normalizing wall stress, which in turn reduces workload of the heart and improves cardiac performance. In accordance with Laplace law, reduction in LV intrinsic diameter reduces wall tension and improves systolic performance (224,225). The effects of PLV were assessed using pressure-volume loops (226). PLV was associated with increased EF, reduction in peak wall stress, increased synchronization in contraction and relaxation, resulting in efficient cardiac performance. The operation was associated with number of drawbacks such as: (a) the mortality rate ranges between 10% and 25%, (b) outcome of the procedure is unpredictable as there are no fixed patient selection criteria, and (c) although systolic function improved, diastolic function deteriorated postoperatively (227,228). Dor combined septal exclusion with endoventriular patch plasty repair and showed to have more beneficial effects than classic PLV (229). MR that occurs due to the abnormality in movements of LV and displacement of papillary muscles has worse prognosis by exacerbating symptoms of CHF. Infarcted areas are of 2 types, akinetic and dyskinetic. Akinetic segments have thin rim of viable myocardium and do not move during systole while dyskinetic segments are transmural LV infarcts with thin ventricular wall leading to true LV aneurysms (230). The aim of SVR is to exclude these akinetic and dyskinetic segments by reducing volume in anterior and septal portions and there by to restore LV function and elliptical contour of LV. SVR is commonly performed in conjunction with CABG and mitral annuloplasty.

b. Coronary Artery Bypass Graft (CABG) Surgery:

The patient who may require CABG is evaluated by extensive diagnostic screening. Patients who show ischemic or hibernating myocardium in association with hemodynamically evident coronary stenoses, preserved RV function, minimal MR, LVEF of <20% and no major associated medical problems are ideal candidates for CABG (231). Low-dose dobutamine echocardiography or positron emission tomography is used to identify and quantify preoperatively areas of hibernating myocardium (232). In patients with an extensive viable akinetic hibernating myocardium with global ventricular dysfunction, CABG is strongly indicated (233). CABG can also be considered when the patient presents with documented ischemia associated with dyspnea and angina because this procedure is considered to prevent further myocyte deterioration and improve myocyte function.

c. Correction of Mitral Regurgitation:

Although LV restoration is considered gold standard procedure to reverse remodeling by removal of a portion of the LV wall in acute stages of HF, correction of MR that was first proposed by Bolling et al (234) is known to reverse the LV remodeling in chronic stages. Hemodynamically, MR is known to worsen the prognosis of patients with CHF (235). Mitral valve repair is often associated with PLV. Presently, it is common procedure to perform mitral valve repair in dilated cardiomyopathic ventricles with severe MR, LVEF <30% and NYHA grade III-IV HF (236). Mitral insufficiency results from enlargement of the mitral annular ventricular apparatus with significant loss of valve leaflet coaptation (237-239). Often there occurs ventricular dilation due to functional restriction of the posterior leaflet.

In patients with dilated cardiomyopathy, mitral insufficiency is considered to be a predictor of low survival (240). The factors on which the severity of MR depends are cardiac geometry, preload and afterload; however, in ischemic dilated cardiomyopathy, the single main determinant of MR is the valvular apparatus, itself. Following an ischemic attack, an extensive geometrical change of the mitral valve occurs, which in turn causes insufficient coaptation of leaflets and MR. Ischemic MR usually results from changes that occur in ventricular geometry, annular dilation and papillary muscle dysfunction.

Mitral valve repair is considered in dilated cardiomyopathy for a morphologically intact but physiologically insufficient mitral valve, whereas mitral annuloplasty leads to readaptation and coaptation of mitral leaflets (236-238). Mitral annuloplasty undersize the mitral annulus by complete encirclage and thereby correct MR. This procedure helps to reverse the spherical shape of the LV contour (238). When leaflet coaptation is >1cm, annuloplasty may not be successful and addition of central "edge-to-edge" stitch abolishes MR by creating a double orifice mitral valve (241). Mitral valve replacement should be considered when the repair is impossible and performed by preserving subchordal attachments, which maintain ventricular geometry and function (242-245). It should be mentioned that whenever patients with CHF present with atrial fibrillation, which may be paroxysmal or chronic that worsens the symptoms, surgical ablation of atrial fibrillation should be considered in such patients (246,247).

d. Diastolic Support:

Passive diastolic support is now considered as an adjunct to LV aneurysectomy and mitral valve repair. Dynamic cardiomyoplasty is a procedure in which conditioned

skeletal muscle, such as latissimus dorsi, is pedunculated and wrapped around the failing heart. The main drawback of this procedure is that muscle may undergo fatigue and thus deteriorate the systolic function. Nevertheless, patients had some clinical benefit, which was attributed to the girdling effect of wrapped muscle, which reduces wall stress, and prevent adverse ventricular remodeling (248). Improvement of diastolic function was associated with interrupting many changes including Ca^{2+} release, changes in ECM, maladaptive gene expression and myocardial apoptosis (249).

Acorn CorCap is a polyester mesh that is wrapped around the ventricle and thus has a girdling effect on the heart as it provides both flexibility and strength. It is useful in dilated cardiomyopathy and allows it to return to elliptical shape thus decreasing mortality (249). This procedure is performed with concomitant valve repair or CABG. It prevents further dilation and thus reverses cardiac remodeling. Myosplint is another external device, which consist of implantable transventricular splint and two epicardial pads (250). The two-epicardial pads are located on the heart. The splint passes through the heart and connects the pads. The splints are tightened to create two smaller LV chamber and thus a bilobular shape. The pads are adjusted to decrease LV intrinsic diameter. In accordance with Laplace law, wall tension is further decreased which in turn decreases wall stress, as well as improve hemodynamics and reverse cardiac remodeling.

e. Left Ventricular Assist Devices:

Left ventricular assist device (LVAD) is considered a therapeutic strategy to improve cardiac function and reverse cardiac remodeling. It usually acts as a bridge for HF patients awaiting cardiac transplantation (251). It induces profound cardiac unloading immediately after its implantation, decreasing both preload and afterload, which are

reflected by decreases in left ventricular end-diastolic volume and increases in relative wall thickness (252).

It is also known that LVAD decreases neurohormonal activation including RAAS, SNS, and the AVP and natriuretic peptide systems (253,254). It has also been reported to decrease myocyte size (253,255-258). LVAD also improves myocardial function as measured in isolated myocytes and muscle strips. A clinical study conducted by Frazier et al (259) showed that LVAD support increased LVEF from 11 ± 5% to 22 ± 17%. At the molecular level, LVAD support induced reversal of genetic expression for SR ATPase, RyR and Na^{+}-Ca^{2+} exchange (260). Electrophysiologically, there was secondary decrease in QTc interval in isolated human ventricular myocytes after LVAD implantation (261).

f. Cardiac Resynchronization Therapy:

Cardiac Resynchronization Therapy (CRT) is known to improve the symptoms and reduce hospitalizations in patients with CHF and intraventricular conduction delay (262-267). Ventricular conduction delays extend MR, reduce LVEF and prolong the pre-ejection time (268,269). In fact, ventricular conduction delays have worst prognosis in patients with CHF (270), and are most frequently due to a left bundle branch block, which is often associated with delayed contraction of LV lateral wall (271).

Atrial sequential left or biventricular pacing in hearts with LBBB can resynchronize the ventricular activation and thus restore the normal contraction by pre-excitation of the left lateral wall (272). CRT can produce the early activation of papillary muscle and can decrease MR as evidenced by a decrease in capillary wedge pressure and an increase in systolic blood pressure when pacing is done from the left lateral wall from the proximity of posterior papillary muscle (273).

II. STATEMENT OF THE PROBLEM AND HYPOTHESES TO BE TESTED

Despite the recent clinical advances in medical and surgical interventions for CHF of ischemic etiology, CHF secondary to MI is still a major cause of death worldwide. Ventricular remodeling is a maladaptive response in CHF and its attenuation is therefore, a principal therapeutic goal (274). CHF model of rats secondary to MI induced by left coronary artery ligation is commonly used as experimental model depicting the alterations similar to those seen in chronic HF in humans (275,276). In this model, hemodynamic measurements made at 8 weeks post-MI showed depressed cardiac function as evidenced by a marked increase in left ventricular end-diastolic pressure (LVEDP) and decrease in mean arterial pressure (MAP) and cardiac contractility (± dP/dt max). This hemodynamic abnormality was associated with structural changes including increases in heart weight to body weight ratio and right ventricular weight to body weight ratio. Since ventricular remodeling is an important predictor of clinical outcome after MI (11,13,277), successful pharmacologic interventions of CHF appear to be associated with attenuation of the LV remodeling process and to decrease mortality in CHF (278,279). In spite of the pharmacological and surgical interventions, many patients continue to experience a progressive decline in cardiac function. Therefore, newer strategies to attenuate ventricular remodeling and function in ischemic heart failure are needed.

CHF is considered as a prothrombotic state in which patients are at increased risk of MI, stroke and venous thromboembolism (280,281,282). These events can lead to sudden death (283) due the arrhythmias. In CHF, antiplatelet agents are used to prevent the aggregation of platelets and commonly used agents include aspirin, clopidogrel and

ticlopidine. In CHF patients who undergo percutaneous transluminal coronary angioplasty (PTCA), the main concern is restenosis of coronary vessels, which is seen in up to 30% to 40% of patients (284). The commonly used antiplatlet agents like aspirin and ticlopidine in CHF have several adverse effects in patients. Clinical trials have demonstrated that aspirin decreases the efficiency of ACE inhibitors in CHF (285,286). Aspirin is also known to have other effects like vasoconstriciton (287), which are mediated by inhibiting cyclo-oxygenase and therby prostacyclin synthesis (288). Recent clinical trials with aspirin and ticlopidine combination was shown to prevent restenosis in coronary vessels after PTCA (289,290) but the main drawback with ticlopidine is that it is known to cause neutropenia and abnormal liver function (291,292). The effects of antiplatlet agents in reversing structural and subcellular remodeling in CHF are not known. Sarpogrelate (SAR), a (5-hydroxy tryptamine) $5HT_{2A}$ receptor antagonist and cilostazol (CIL), a phosphodiesterase-III (PDE-III) inhibitor are newer antiplatelet and antithrombotic agents belonging to two different drug groups and share many pharmacological effects in common. Clinical trials with these agents have shown beneficial effects in preventing restenosis of coronary vessels after stent implantation as well as in preventing platelet aggregation in peripheral vascular disease. We hypothesized that if these agents could also prevent detrimental ventricular remodeling in CHF, it would be beneficial for the patients to use a single drug that can prevent platelet aggregation as well as ventricular remodeling in CHF rather than using multidrug therapy. Thus, the aim of this study was to examine the effects of these antiplatelet agents, SAR and CIL, on LV function and subcellular remodeling, as well as on electrocardiographic (ECG) changes and mortality in post-MI rats. In this study, we

examined a model of MI induced by left coronary artery ligation in the rat, first to correlate the hemodynamic and pathological changes with myofibrillar and SR protein content and gene expression, and next to examine the effects of $5HT_{2A}$ receptor blockade with SAR and PDEIII inhibition with CIL on these alterations.

Myofibrillar Ca^{2+}-stimulated ATPase activity is a major parameter, which determines the ability of cardiac muscle to generate contractile force (293). Furthermore, different amounts of myosin heavy chain (MHC) isoforms i.e., α-MHC and β-MHC determine the myofibrillar Ca^{2+} stimulated ATPase activity (294,295). In CHF, a shift in composition of myosin isoforms has been reported to depress myofibrillar Ca^{2+}-stimulated ATPase activity resulting in myocardial contractile dysfunction (295-300). This study was thus undertaken to examine the pattern of changes in MHC isoforms, mRNA levels for α-MHC and β-MHC and myofibrillar Ca^{2+} stimulated ATPase activity in a rat model of CHF. We tested the effects of antiplatelet agents on myofibrillar Ca^{2+}-stimulated and Mg^{2+} ATPase, MHC isoforms and gene expression in infarcted hearts.

SR plays a significant role in the regulation of contraction and relaxation and also in regulating intracellular concentration of Ca^{2+} in cardiomyocytes, by its ability to release and store Ca^{2+} (301,138,302). Ca^{2+} release channel, ryanodine receptor (RyR) and Ca^{2+} pump ATPase (SERCA2a) mediates SR Ca^{2+} release and accumulation, while phospholamban (PLB) regulates the SR Ca^{2+} pump activity (138,302,303). Previous studies in both humans and animals, on SR have scattered information on SR Ca^{2+} release and Ca^{2+} uptake activities as well as SR protein content and gene expression in CHF (302-310). In CHF, there occurs a significant alteration in SR protein content and SR Ca^{2+} release and Ca^{2+} uptake activities, which in turn results in Ca^{2+} handling

abnormalities in cardiomyocytes and cardiac dysfunction (302-304,311). In failing hearts, although the alteration in SR gene expression has been shown to modify the molecular structure of SR protein (303), the mechanism of remodeling of SR needs further elucidation. In CHF, previous studies have shown that alteration in SR Ca^{2+} uptake and Ca^{2+} release activities as well as changes in SR protein content and gene expression for SERCA2a and PLB is related to LV dysfunction and abnormalities in Ca^{2+} handling by cardiomyocytes (312,152,313,153,314,315). An improvement of altered SR function is a possible mechanism for the benefit of patients with CHF. However, there are no studies investigating the effects of antiplatelet agents on cardiac SR function in animals with CHF.

As previous studies have shown that $5HT_{2A}$ receptor blockade with Ketanserin and PDEIII inhibition with milrinone and amrinone can prevent cardiac remodeling and improve heart function in CHF due to MI, it is likely that ventricular remodeling, myofibrillar and SR remodeling in the failing heart is prevented by these antiplatelet drugs, SAR and CIL. Accordingly it is planned to examine the effects of SAR and CIL treatment in infarcted rats on LV function, myofibrillar Ca^{2+}-stimulated ATPase activity, MHC protein content and gene expression, SR Ca^{2+} uptake and release, SR protein content as well as changes in SR gene expression.

III. MATERIALS AND METHODS

A. Experimental Model

All experimental protocols were approved by the Animal Care Committee of the University of Manitoba and follow the guidelines established by the Canadian Institutes of Health Research. MI was induced in male Sprague–Dawley rats (175–200 g) by occlusion of the left coronary artery as described earlier (139). Briefly, the heart in anesthetized animals was exposed through left thoracotomy and the left coronary artery was ligated at about 2 mm from its origin at the aorta. The heart was repositioned in the chest and the incision was closed with a purse string suture. Sham-operated rats were treated in the same way except that the artery was not ligated. Mortality of experimental rats was approximately 35 % within 48 hr.

B. Protocol for Drug Treatments

All rats received standard care, kept at 12 hr day/night cycle and were fed rat chow and water *ad libitum*. Three weeks after the operation, animals were assigned to control, untreated infarcted (MI), 5 mg/kg SAR-treated infarcted, 5 mg/kg CIL-treated infarcted group. All drugs were given daily for 5 weeks via a gastric tube starting at 3 weeks after induction of MI; control animals received vehicle alone. A flow chart showing drug treatments is shown in Figure 1.

C. Hemodynamic Studies

Hemodynamic measurements were carried out at 8 weeks post-surgery as described previously by Dixon et al. 1990 (139). Briefly, animals were anesthetized with an intraperitoneal injection of a mixture of ketamine (90 mg/kg) and xylazine (10 mg/kg). The right carotid artery was exposed and cannulated with a microtip pressure transducer (model SPR-249, Millar Instruments, Houston, TX, USA). The catheter was advanced carefully through the lumen of the carotid artery, until it entered the LV, and then was secured with a silk ligature around the artery. Measurements of LV systolic pressure (LVSP), LVEDP, heart rate, rate of pressure development (+dP/dt) and rate of pressure decay (-dP/dt) in anesthetized animals were performed using AcqKnowledge software (3.0.3 MP100, BIOPAC System Inc., Goleta, Calif). After hemodynamic measurements, the abdominal aorta was cannulated and 8-10 ml blood was collected into EDTA-containing ice-chilled vacutainers for catecholamine estimation. Blood samples were centrifuged for 10 min at a speed of 3,000 rpm at 4 °C and the plasma was collected and stored at -70 °C for further analysis. The hearts were removed, the atria and the large vessels were carefully trimmed, and the ventricles were separated and weighed. The LV (including septum) as well as the scar tissue were dissected and weighed. The lungs and the liver from all the animals were also removed and weighed for wet weight; then they were dried in the oven at 60 °C for 48 h and weighed again for dry weight.

D. Echocardiography

The echocardiography was performed at baseline (i.e. 3 weeks post-surgery) and after 5 weeks of treatment (i.e. 8 weeks post-surgery). Transthoracic 2D-guided M-mode

echocardiographic tracings were recorded as described previously (316). Briefly, rats were anesthetized with isoflurane inhalation. Echocardiograms were performed using an ultrasound imaging system (Agilent SONOS 5500) equipped with a S12 phased-array transducer (Agilent Technologies Inc.). A 2-dimensional short-axis view of the LV cavity was obtained at the level of the papillary muscles. M-mode tracings were recorded through the anterior and posterior LV walls at a speed of 100 mm/s. Global LV systolic function was assessed by calculating LV fractional shortening (FS) and LVEF using the formula (LV intrinsic diastolic diameter –LV intrinsic systolic diameter) x 100 / LV intrinsic diastolic diameter and (LV intrinsic diastolic diameter^3 –LV intrinsic systolic diameter^3) x 100 / LV intrinsic diastolic diameter^3, respectively (316).

E. Electrocardiography

Electrocardiograms (ECGs, limb leads I, II, III, aVR, aVL and aVF) were recorded at the time of surgery, 24 hours post-surgery, 3 weeks post-surgery and after 5 weeks of treatment (i.e. 8 weeks post-surgery). ECGs were recorded for 5 to 10 min using AcqKnowledge 3.0.3 software for Windows (BIOPAC SYSTEM Inc., Goleta, Calif) on stabilized animals under isoflurane inhalational anesthesia. RR, PQ (or PR when Q wave was not present) and QT intervals were measured. QT intervals were measured from the onset of Q waves until termination of the T waves. Onset of the R wave was used if Q wave was not present. QT was corrected for the heart rate (HR) using Bazett's formula (QT_c = QT/square root of RR interval). RR interval immediately preceding the QT interval was used to correct for heart rate. QRS complex duration was not analysed because QRS complex/T wave transition was ill defined in the recordings.

Pathological Q waves (a negative deflection at least 25 mV in amplitude, preceding the R wave) or QS complexes (in the absence of R wave) were considered as reliable ECG signs of a definite MI in the chronic stage.

F. Infarct Size Estimation

To determine the extent of myocardial necrosis, scar size in the LV was measured histologically. The heart was excised, and sliced transversely from apex to base into 2 mm thick sections. The sections were incubated in a 1 % solution of triphenyl-tetrazolium chloride (TTC) for 20 min at 37^0 C. The tissue sections were then fixed in a 10% formalin solution and weighed. Normal viable myocardium was stained dark red. Infarcted myocardium fails to stain with TTC. The scar was calculated as % of the LV wall area using the formula:

$$\text{Infarct size/LV mass (\%)} = \frac{\Sigma \text{ Infarct weight}}{\Sigma \text{ Total LV weight}} \times 100$$

G. Determination of Myofibrillar Mg^{2+}-ATPase and Ca^{2+} stimulated ATPase Activities

The myofibrillar fraction was isolated as described by Solaro et al. (317) and was suspended in a final solution containing 100 mM KCl, 20 mM Tris-HCl (pH 7.0). Myofibrillar Mg^{2+}-ATPase and Ca^{2+}-stimulated ATPase activities were determined according to methods used previously in our laboratory (318). Mg^{2+}-ATPase activity was measured at 30°C in a medium containing 20 mM imidazole (pH 7.0), 2 mM $MgCl_2$, 2 mM Na_2ATP, 10 mM NaN_3, 1.6 mM ethyleneglucol-bis (β-aminoethylether) N, N, N', N'-tetraacetic acid (EGTA) and 50 mM KCl. Total ATPase activity was determined in

the same medium except that EGTA was replaced by 10 μM of free Ca^{2+}. Ca^{2+}-stimulated ATPase activity was taken as the difference between values obtained for total and Mg^{2+}-ATPase activities. All reactions were terminated at 5 min by the addition of 1 ml of 12 % trichloroacetic acid. These samples were centrifuged at 1000 X g and the phosphate in the protein-free supernatant was determined by colorimetric method.

H. Analysis of Cardiac Myosin Heavy Chain Isoforms

Cardiac MHC isoforms were determined under denaturing conditions. The α-and β-MHCs were separated on a 4 % polyacrylamide gel as described previously (319). Equal amount of protein sample (2-4 μg protein) was loaded in each well. The electrophoresis was carried out at a constant 220 V for 3-3.5 hr with cooling between 13°C and 17°C and continuous stirring of the buffer. The gels were stained with Coomassie brilliant blue R250 for 2 hr and were destained with 7 % acetic acid by diffusion. The relative amount of isoforms was estimated by Imaging Densitometer (GS-800, Bio-Rad).

I. Determination of SR Ca^{2+}-uptake and SR Ca^{2+}-release activities

SR vesicles were obtained using a method described previously (320–322, 323). LV tissue was pulverized and homogenized in a buffer (10 ml/g tissue) containing (in mM) 10 $NaHCO_3$, 5 NaN_3, and 15 Tris HCl (pH 6.8) with a Polytron homogenizer (Brinkmann, Westbury, NY). The homogenate was centrifuged for 20 min at 11,000 g. The pellet was discarded, and the supernatant was further centrifuged for 45 min at 43,000 g (JA 20 rotor; Beckman). The resultant pellet was resuspended in a buffer

containing 0.6 M KCl and 20 mM Tris HCl (pH 6.8) and centrifuged for 45 min at 43,000 g. The final pellet containing the SR fraction was suspended in a buffer containing 250 mM sucrose and 10 mM histidine (pH 7.0), aliquoted, and frozen in liquid nitrogen before being stored at -80°C. All buffers used for isolation contained a cocktail of protease inhibitors consisting of aprotinin, leupeptin, 4-(2-aminoethyl) benzenesulfonyl fluoride, and 0.1% phenylmethylsulfonyl fluoride to prevent any degradation of proteins during the isolation procedure.

SR Ca^{2+} uptake was measured using a procedure described previously (320–322, 323). The reaction mixture contained (in mM) 50 Tris-maleate (pH 6.8), 5 NaN_3, 5 ATP, 5 $MgCl_2$, 120 KCl, 5 potassium oxalate, 0.1 EGTA, 0.1 $45CaCl_2$ (20 mCi/l), and 25 μM ruthenium red. The initial free Ca^{2+} concentration in this medium, determined using the program of Fabiato (324), was 8.2 μM. Ruthenium red was added as an inhibitor of the Ca^{2+} release channel. The reaction was initiated by adding SR vesicles (20 μg protein) to the reaction mixture at 37°C and was terminated after 1 min by filtration. The filters were washed, dried, and counted in a beta scintillation counter. SR Ca^{2+} uptake at 1 min was found to be in the linear range.

The Ca^{2+} release activity of SR vesicles was determined by a method described earlier (325). Briefly, the SR fraction was incubated with 10 μM $45CaCl_2$ (20 mCi/ml) and 5 mM ATP for 45 min in a medium containing (in mM) 100 KCl, 5 $MgCl_2$, 5 NaN_3, 20 Tris HCl (pH 6.8), and 5 potassium oxalate. The Ca^{2+} release was induced by addition of 1 mM EGTA and 1 mM Ca^{2+} to the reaction mixture and terminated at 15 s by Millipore filtration. Radioactivity in the filter was counted in 10 ml of scintillation fluid by using a

liquid scintillation counter (Beckman Instruments). Treatment of the SR preparation with 20 μM ryanodine prevented 95–100% of this Ca^{2+} induced Ca^{2+} release.

J. Relative Content of SR Ca^{2+}cycling Proteins

The relative protein content of SR Ca^{2+}cycling proteins, SERCA2a, RyR as well as PLB and its phosphorylated form, serine[16] PLB, were determined by Western blot analysis as described previously (321,322,323). SR samples (20 μg) were separated by SDS-polyacrylamide gel electrophoresis on a gradient gel (4-20 %) (for RyR), 10 % (for SERCA2a) and 15 % (for PLB and serine[16] PLB) gels and transferred to polyvinylidene difluoride membranes. Membranes were probed with a monoclonal anti-RyR antibody and monoclonal anti-SERCA2a antibody obtained from Affinity Bioreagents (Golden, CO), as well as monoclonal anti-PLB polyclonal antibody obtained from Upstate Biotechnology and polyclonal anti-serine[16] PLB antibody obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Appropriate secondary antibodies were used, and the antibody-antigen complexes in all membranes were detected using the ECL kit (Amersham Life Science, Oakville, ON, Canada). An imaging densitometer (model GS-800; Bio-Rad, Hercules, CA) was used to scan the protein bands, and data were quantified using Quantity One 4.4.0 software (Bio-Rad).

K. Isolation of Total RNA and Northern Blot Analysis

Total RNA was isolated from the viable LV (including the septum) of sham control and experimental rats with or without drug treatment by the acid guanidinium thiocyanate-phenol-chloroform method (TRIzol Reagent, GIBCO-BRL Life

Technologies, Burlington, ON, Canada), as described previously (326,327). Briefly, frozen samples of viable LV were ground with a pestle and mortar and homogenized with a Polytron (model PT3000) at 12,000 rpm twice for 15 s each, with 20 s between homogenizations, in the presence of 1.5 ml of TRIzol Reagent. The mixture was cooled on ice for an additional 15 min and centrifuged at 12,000 g (model J2-HS, Beckman Instruments) for 10 min at 4°C. The supernatant was incubated with chloroform (0.3 ml/sample) for 5 min at room temperature and then centrifuged at 12,000 g for 15 min at 4°C. The RNA containing the upper aqueous phase was kept at - 20°C for 4 hr after addition of 0.75 ml of isopropyl alcohol. After centrifugation at 12,000 g for 10 min at 4°C, RNA pellets were suspended in 75% molecular biology-grade ethanol diluted with diethyl pyrocarbonate (DEPC)-treated water. After sedimentation at 12,000 g for 10 min at 4°C, RNA pellets were washed again with 75% ethanol, centrifuged at 12,000 g for 10 min at 4°C, and vaccum dried by Speed Vac (model SC110, Sarvant Instruments, Farmingdale, NY). Samples were dissolved in DEPC-treated water, and the RNA concentration was calculated from the absorbance at 260 and 280 nm with SPECTRAmax PLUS (Molecular Devices, Sunnyvale, CA). The final RNA pellet was resuspended in sterile distilled water containing 0.1% DEPC and stored at - 70°C. Total RNA (20 μg) was denatured at 65°C for 10 min and size fractionated on a 1.2% agarose gel containing 1.1 M formaldehyde. The blotted samples were transferred onto nylon membranes (Schleicher & Schuell, Keene, NH), UV cross-linked, and hybridized to randomly primed Cdna

L. Data Analysis

15-20 animals were randomly selected for data analysis from each experimental group. Data are expressed as means ± S.E.M. Statistical differences of the mean values were evaluated by Student's t-test, paired or unpaired as appropriate, and one-way ANOVA at a level of significance $P < 0.05$. Mortality was analysed using Kaplan Meier survival analysis.

IV. RESULTS

A. Mortality in Untreated and Drug-treated Rats

Out of 210 rats that underwent coronary ligation or sham operation, 71 rats died within the first 48 hours after surgery, which corresponds to 34 % mortality. 5 rats died in untreated MI group during 48 hr post surgery to 21days. We lost 10/36 rats during the 3-8-week post-operative (treatment-equivalent) period in the untreated MI group, 4/35 animals died in the SAR group and 13/34 animals died in the CIL group. There was no animal loss in the sham group of 29 rats. The data on mortality, as well as Kaplan Meier Survival plot for all groups is shown in Figure 2.

B. General Characteristics, Infarct Size and Cardiac Contractile Function

The general characteristics of sham-operated, MI-untreated, SAR-treated and CIL-treated animals are shown in Table 1. Body wt was significantly decreased in untreated MI group compared to control animals. Untreated MI rats had clinical signs of CHF, i.e. lung congestion as reflected by the increased lung wet/dry wt ratio, ventricular chamber enlargement, RV hypertrophy, pulmonary edema and ascites. Ligation of the coronary artery resulted in scar formation with well-defined borders (Figure 3); however, no change in infarct size among different group was seen. Treatment with SAR and CIL led to significant decrease in total ventricular wt, RV wt, ventricular/body wt ratio and lung wet/dry wt ratio. There was no change in scar wt irrespective of treatment since the treatment was started in the chronic stage of MI when the scar is already formed (Table 1).

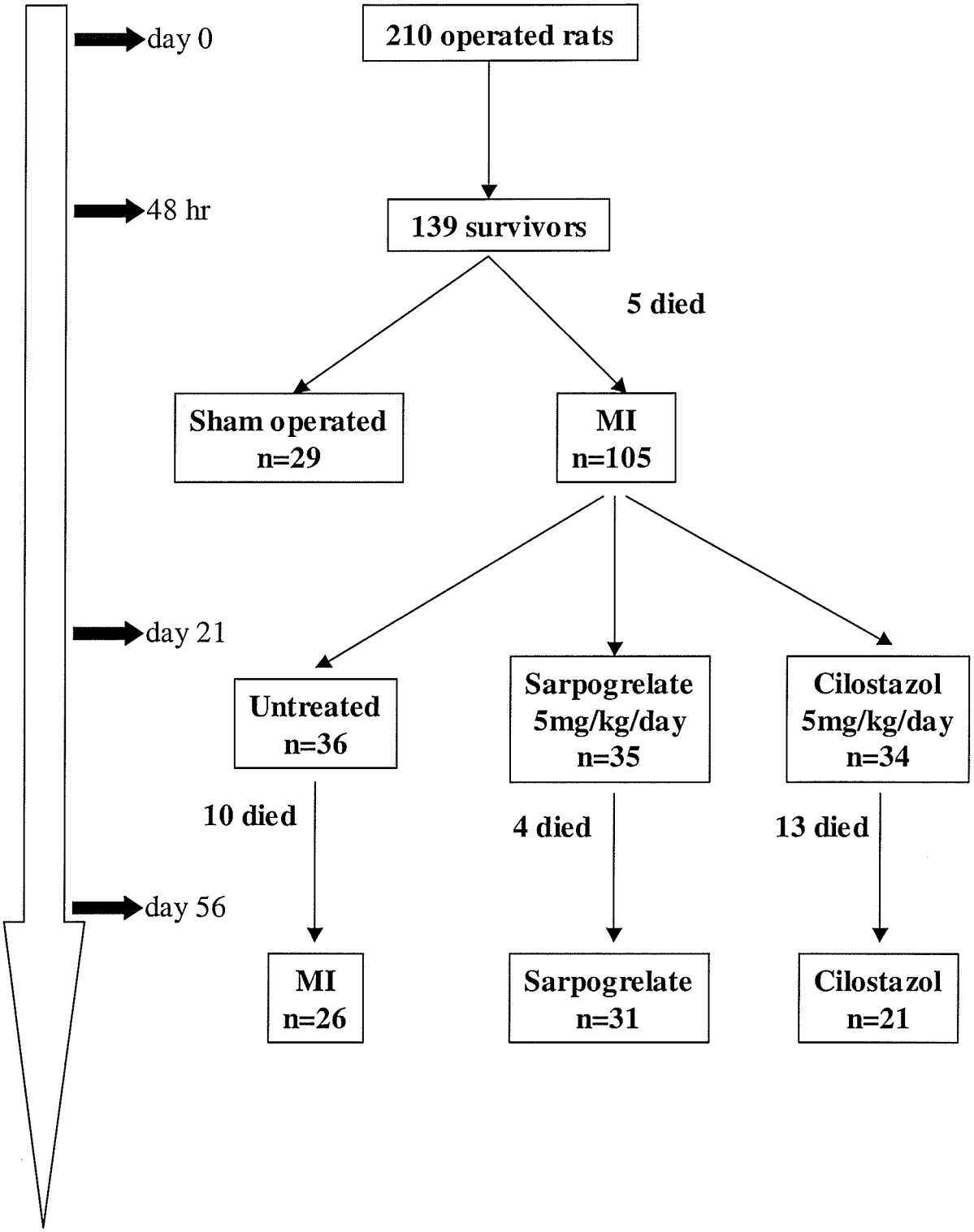

Figure 1. Flow diagram of various groups of rats according to the presence of MI and treatment with SAR or CIL

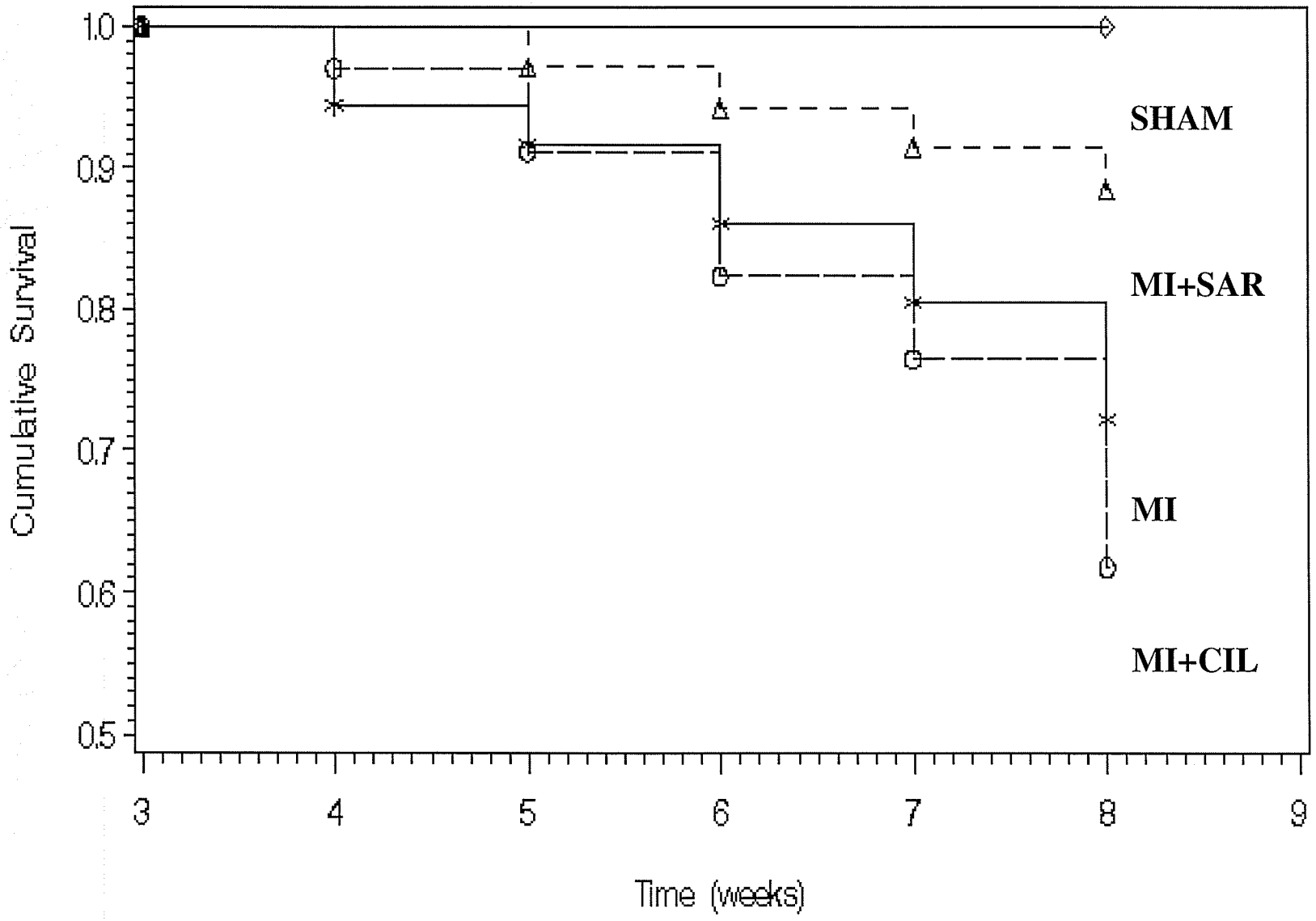

Figure 2. Kaplan Meier Survival Curves of sham, MI, SAR-treated and CIL-treated groups

Table 1
General characteristics of sham, MI, SAR-treated and CIL-treated groups

	SHAM	MI	MI + SAR	MI + CIL
Body weight (g)	573 ± 14.57	510 ± 8.99 *	529 ± 4.76	524 ± 10.60
Ventricular wt (mg)	1.31 ± 0.03	1.52 ± 0.04 *	1.39 ± 0.04 #	1.38 ± 0.03 #
Ventricular wt/Body wt (mg:g)	2.38 ± 0.02	3.03 ± 0.09 *	2.71 ± 0.08 #	2.77 ± 0.08 #
RV (mg)	0.28 ± 0	0.43 ± 0.03 *	0.32 ± 0.01 #	0.31 ±0.02 #
Scar wt (mg)	---	0.17 ± 0.01	0.16 ± 0.01	0.16 ± 0
Infarct size/LV mass (%)	---	19.86 ± 2.31	19.53 ± 1.74	19.46 ± 0.42
Lungs wet/dry ratio	4.52 ± 0.05	5.09 ± 0.09 *	4.82 ± 0.04 #	4.79 ± 0.07 #
Liver wet/dry ratio	3.17 ± 0.02	3.27 ± 0.04	3.25 ± 0.05	3.24 ± 0.02

Values are means ± SE; n=15-20 animals for each group. MI, myocardial infarction; LV, left ventricle; RV, right ventricle; SAR, Sarpogrelate; CIL, Cilostazol. * $P<0.05$, significantly different from sham control group. # $P<0.05$, significantly different from MI group.

Figure 2. Triphenyl Tetrazolium Chloride (TTC) stained sections of sham, MI, SAR-treated and CIL-treated groups

Hemodynamic parameters obtained from the different experimental groups 8 weeks after surgery are shown in Table 2. Infarcted animals had significantly higher heart rate and developed LV dysfunction with decreased LVSP, MAP, + dP/dt, - dP/dt and elevated LVEDP compared to control animals. The LVEDP was reduced, whereas the ± dP/dt, LVSP and MAP were increased towards sham levels in SAR-treated and CIL-treated groups compared with untreated MI. Heart rate was significantly increased in untreated-MI. Although SAR and CIL treatments decreased the heart rate compared to MI rats, the values did not reach statistical significance.

C. Alterations in Echocardiographic Parameters

In order to obtain information on the myocardial contractile state, cardiac function was assessed by echocardiography and compared with the control models. Figure 4 shows representative 2D and M-mode echocardiographic tracings from each experimental group. By 3 weeks after surgery, all ligated animals developed severe systolic dysfunction evidenced by a dramatic 50 % drop in EF (Table 3). In the following 5 week period, EF values did not show worsening or improvement in the untreated-MI group. As shown in Figure 4 and Table 3, contractile dysfunction in these animals was accompanied by an increase in both systolic and diastolic LV diameters compared to sham controls. Compared to untreated MI, a 5-week treatment with SAR and CIL significantly improved EF and LV diameters to similar extent.

Changes in CO and SV showed similar tendency as EF. At 8 weeks after coronary artery ligation, CO dropped by 27% and SV by 30% in the absence of antiplatelet drug

Table 2
Hemodynamic parameters of sham, MI, SAR-treated and CIL-treated groups

	SHAM	MI	MI + SAR	MI + CIL
Arterial SP (mm Hg)	135.81 ± 5.59	94.64 ± 2.86 *	118.81 ± 4.47 #	119.65 ± 2.55 #
Arterial DP (mm Hg)	92.45 ± 4.06	61.25 ± 2.3 *	87.07 ± 4.61 #	87.50 ± 3.07 #
MAP (mm Hg)	112.87 ± 3.18	72.37 ± 1.23 *	103.12 ± 4.39 #	100.41 ± 2.70 #
LVSP (mm Hg)	127.58 ± 4.22	88.30 ± 2.30 *	114.59 ± 4.61 #	106.92 ± 2.56 #
LVEDP (mm Hg)	8.31 ± 0.38	19.68 ± 0.46 *	11.59 ± 0.64 #	11.70 ± 0.54 #
+dP/dt (mmHg/sec)	8186 ± 290	3306 ± 118 *	6172 ± 216 #	6280 ± 247 #
-dP/dt (mmHg/sec)	6738 ± 561	2740 ± 105 *	4301 ± 220 #	4529 ± 295 #

Values are means ± SE; n=15-20 animals for each group. MI, myocardial infarction; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; +dP/dt, rate of pressure development; -dP/dt, rate of pressure decay; SAR, Sarpogrelate; CIL, Cilostazol. * $P<0.05$, significantly different from sham control group. # $P<0.05$, significantly different from MI group.

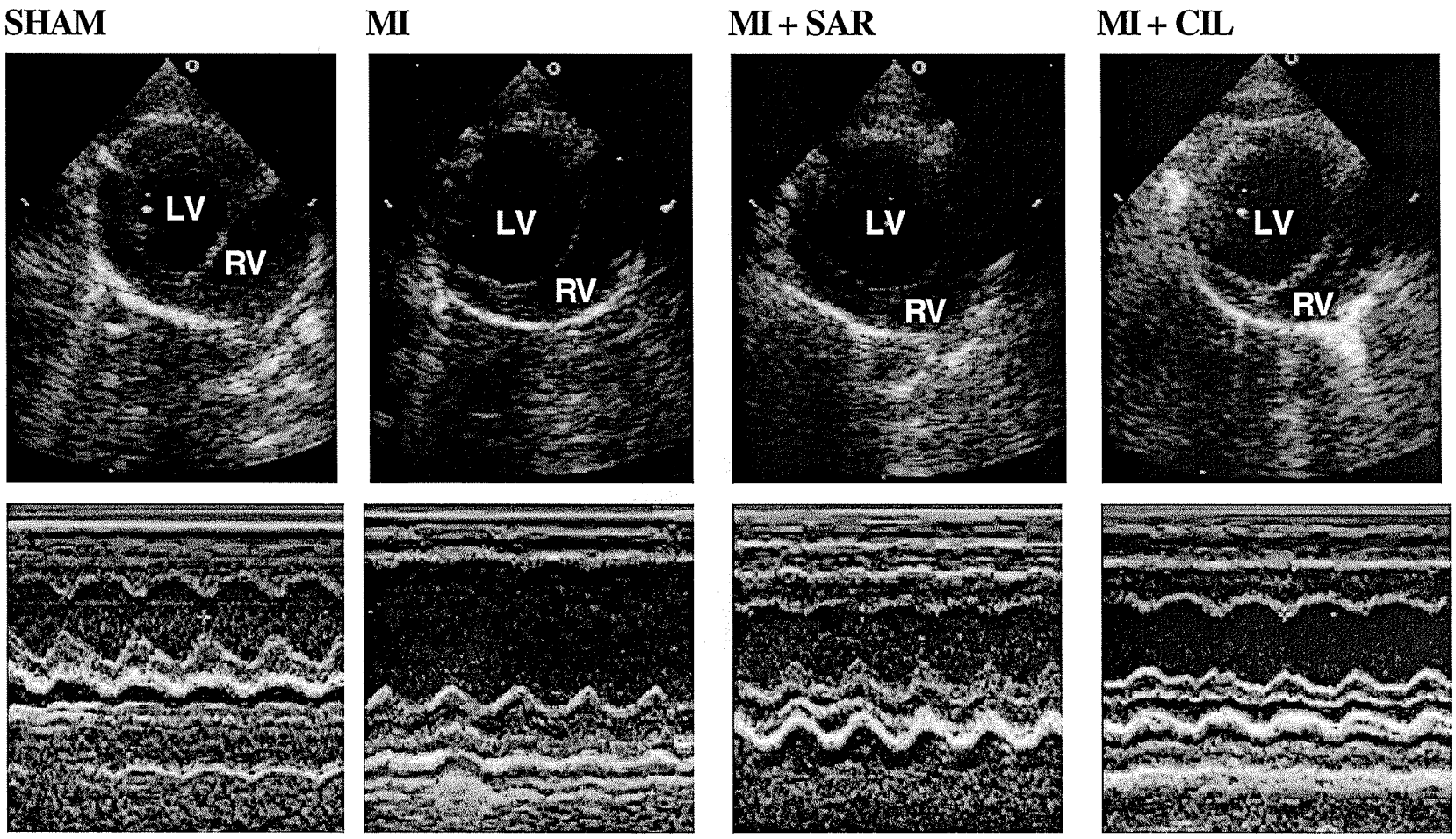

Figure 5. 2D and M-mode images of sham, MI, SAR-treated and CIL-treated groups

treatment. Both SAR and CIL improved CO and SV significantly. Untreated MI rats exhibited a marked increase in LV end-diastolic and LV end-systolic volumes (2.66 ± 0.12 ml and 1.71 ± 0.09 ml, respectively) compared with sham-operated rats (1.02 ± 0.06 ml and 0.22 ± 0.02 ml, respectively). Treatment with SAR and CIL partially restored increased LV volumes to control level.

D. Alterations in Electrocardiographic Parameters

Representative ECG tracings recorded in different experimental groups are shown in Figure 5. The majority of the animals surviving coronary ligation over 48 hr developed pathological Q waves or QS complexes at least in one of the leads of the ECG, indicating the characteristic sign of definite chronic MI. Sham controls were devoid of pathological QRS alterations. As indicated in Table 4, ECGs of failing hearts with chronic MI showed significant increase in PQ (PR), QT and QT_c and decrease in RR intervals compared to controls. Since ECG results of sham animals at 8 weeks after surgery were not significantly different from those measured before surgery, latter data is not shown here. SAR and CIL treatments had no significant effect on PQ and RR intervals in the infarcted animals. QT_c was significantly prolonged in untreated-MI rats when compared to sham controls from (0.179 ± 0.002 sec to 0.216 ± 0.003 sec). Although SAR and CIL groups showed decreased tendency in QT_c when compared to untreated MI, data didn't reach statistical significance. However, CIL treated rats also showed increased incidence of premature ventricular complexes (PVCs) and ventricular tachycardia (VT) (Figure 4), which may be attributed to increased mortality in this group.

Table 3
Echocardiographic measurements of left ventricular volumes and intrinsic diameters

	SHAM	MI	MI + SAR	MI + CIL
$LVID_s$ (cms)	0.43 ± 0.02	0.99 ± 0.01 *	0.68 ± 0.04 #	0.69 ± 0.03 #
$LVID_d$ (cms)	0.77 ± 0.02	1.16 ± 0.01 *	0.93 ± 0.03 #	0.97 ± 0.03 #
LVESV (ml)	0.22 ± 0.02	1.71 ± 0.09 *	0.64 ± 0.09 #	0.62 ± 0.07 #
LVEDV (ml)	1.02 ± 0.06	2.66 ± 0.12 *	1.50 ± 0.13 #	1.69 ± 0.13 #
CO (ml/min)	325 ± 26.5	240 ± 27.1 *	342 ± 30.4 #	314 ± 18.3 #
HR (beats/min)	317 ± 3.70 *	355 ± 7.50 *	340 ± 4.60	348 ± 5.40
SV (ml/min)	1.03 ± 0.09	0.72 ± 0.08 *	1.03 ± 0.09 #	1.04 ± 0.06 #
EF (%)				
Before treatment	83.14 ± 1.16	46.52 ± 2.20 *	49.54 ± 1.25	49.89 ± 2.53
After treatment	80.41 ± 1.20	41.89 ± 1.97 *	63.97 ± 2.71 #	68.61 ± 1.87 #
FS (%)				
Before treatment	45.88 ± 1.31	20.54 ± 1.36 *	21.70 ± 0.74	22.92 ± 0.79
After treatment	44.21 ± 1.19	15.94 ± 0.75 *	32.75 ± 1.10 #	32.77 ± 1.36 #

Values are means ± SE; n=25-30 animals for each group. MI, myocardial infarction; $LVID_s$, left ventricular intrinsic systolic diameter; $LVID_d$, left ventricular intrinsic diastolic diameter; LVESV, left ventricular end-systolic volume; LVEDV, left ventricular end-diastolic volume; LVSV, left ventricular stroke volume; SAR, Sarpogrelate; CIL, Cilostazol. * $P<0.05$, significantly different from sham control group. # $P<0.05$, significantly different from MI group

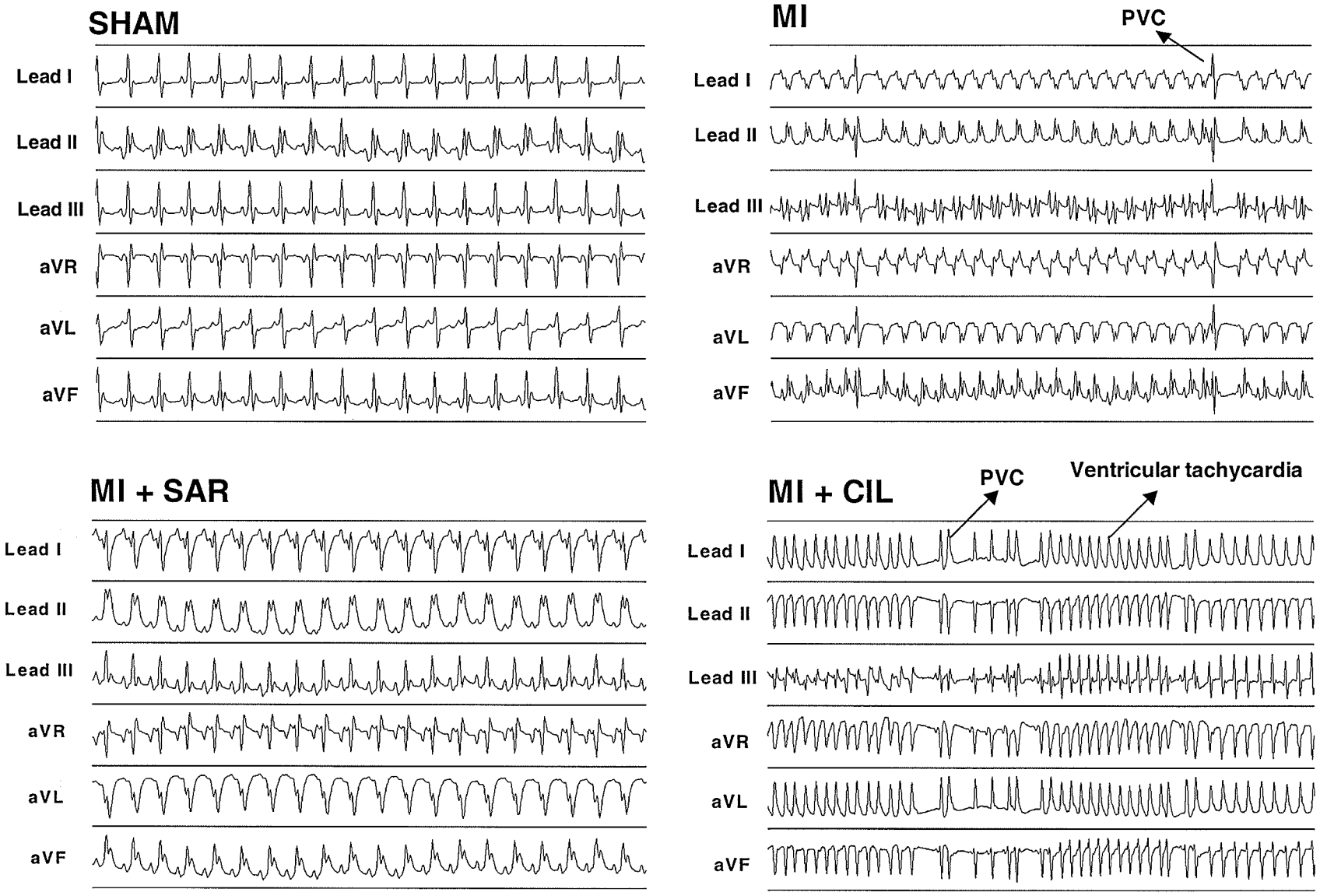

Figure 5. ECG recordings of sham, MI, SAR-treated and CIL-treated groups

Table 4
Electrocardiographic parameters of sham, MI, SAR-treated and CIL-treated groups

	SHAM	MI	MI + SAR	MI + CIL
PR interval (sec)	0.051 ± 0.02	0.057 ± 0.001 *	0.057 ± 0.002	0.056 ± 0.002
QRS duration (sec)	0.033 ± 0.01	0.036 ± 0.001 *	0.034 ± 0.001	0.035 ± 0.001
QT interval (sec)	0.077 ± 0.001	0.090 ± 0.001 *	0.090 ± 0.002	0.085 ± 0.002
RR interval (sec)	0.185 ± 0.003	0.174 ± 0.003 *	0.178 ± 0.004 *	0.173 ± 0.006 *
QT_c	0.179 ± 0.002	0.216 ± 0.003 *	0.213 ± 0.002	0.212 ± 0.004

Values are means ± SE; n=15-20 animals for each group. MI, myocardial infarction; SAR, Sarpogrelate; CIL, Cilostazol.
* $P<0.05$, significantly different from sham control group. # $P<0.05$, significantly different from MI group.

E. Changes in Myofibrillar ATPase Activites

The data in Figure 6 show that myofibrils isolated from the viable LV of infarcted hearts exhibited a lower Ca^{2+}-stimulated ATPase activity (6.07 ± 0.30 μmol Pi/mg/h) compared with sham-operated rats (10.67 ± 0.44 μmol Pi/mg/h, $P < 0.05$). Treatment with SAR and CIL partially normalized the ATPase activity (8.04 ± 0.25 and 8.05 ± 0.54 μmol Pi/mg/h, respectively) (Figure 6). Myofibrillar Mg^{2+}-ATPase activity did not show any significant change in different groups.

F. Myosin Heavy Chain Isoforms

The relative protein content of MHC was determined by using a 4-16 % gradient gel stained with Coomassie stain. No significant alterations in the total protein content level of MHC were observed in rats with MI irrespective of the treatment (data not shown). α- and β-MHC isoforms were separated by using 4 % SDS-PAGE. β-MHC exhibited higher electrophoretic mobility than α-MHC, which was the dominant (> 90 %) isoform in sham group (Figure 7). In the MI group, α-MHC isoform was reduced significantly while that for β-MHC isoform was increased markedly (Figure 7); β-MHC isoform was increased in untreated MI hearts from 27.84 % to 55.98 % of total MHC in the viable tissue of LV whereas α-MHC isoform was decreased from 72.16 % to 44.02 % of total MHC. Antiplatelet drug treatment with SAR and CIL partially prevented the increase in β-MHC as well as the decrease in α-MHC due to MI.

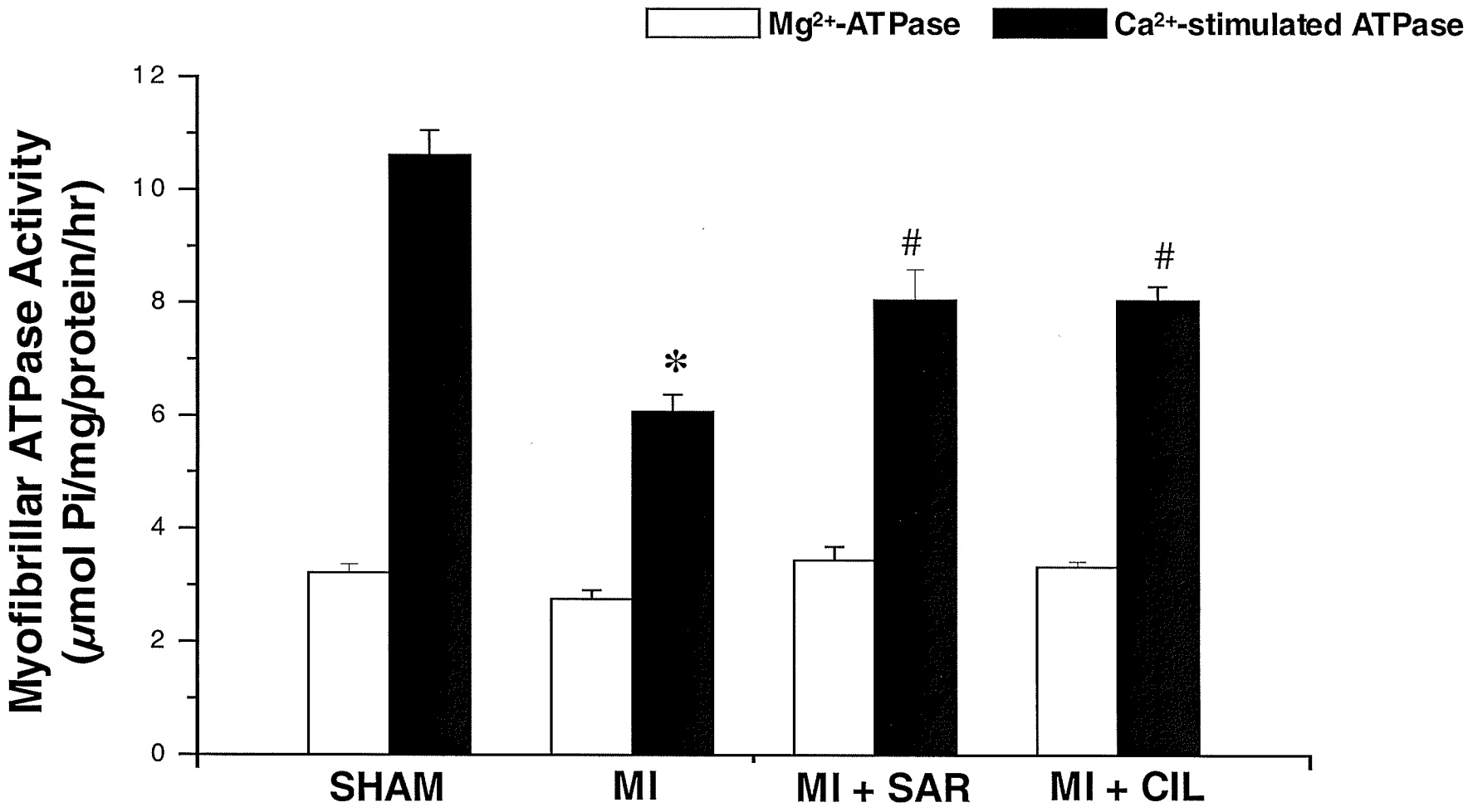

Figure 6. Myofibrillar Ca^{2+}-stimulated ATPase and Mg^{2+}-ATPase activitiesof sham, MI, SAR- treated and CIL-treated groups

G. SR Ca^{2+}-Uptake and Ca^{2+}-Release Activities

Cardiac Ca^{2+} uptake activities were determined in SR vesicles obtained from the viable LV tissue in sham and failing hearts from animals treated with or without antiplatelet agents; results are shown in Figure 8. The SR Ca^{2+} uptake was lower in the MI group compared with the sham control group but was improved significantly upon SAR or CIL treatment. The data for Ca^{2+}release activities of the SR isolated from different experimental groups are shown in Figure 8. As compared with sham, MI markedly decreased the SR Ca^{2+}release activity. Treatment with SAR or CIL prevented decrease in SR Ca^{2+}release activity due to MI.

H. Effects of Sarpogrelate and Cilostazol on SR Protein Contents

To study the molecular mechanisms of the beneficial effects of antiplatelet agents, SAR and CIL on SR Ca^{2+} transport activities in the infarcted heart, alterations in SR protein content were measured in the sham and infarcted animals with or antiplatelet treatment. Protein contents of the LV SR SERCA2a, RyR, PLB and phosphorylated PLB were identified by enhanced chemiluminescence Western blotting (Figures 9 and 10). The SR SERCA2a, RyR, phospholamban, and phosphorylated PLB protein levels were reduced by 71 %, 62 %, 23% and 63 % respectively, in failing LV (Figures 9 and 10). The reduction in protein contents of cardiac SR SERCA2a, RyR, PLB and phosphorylated PLB in the infarcted animals was prevented by SAR and CIL treatment, significantly.

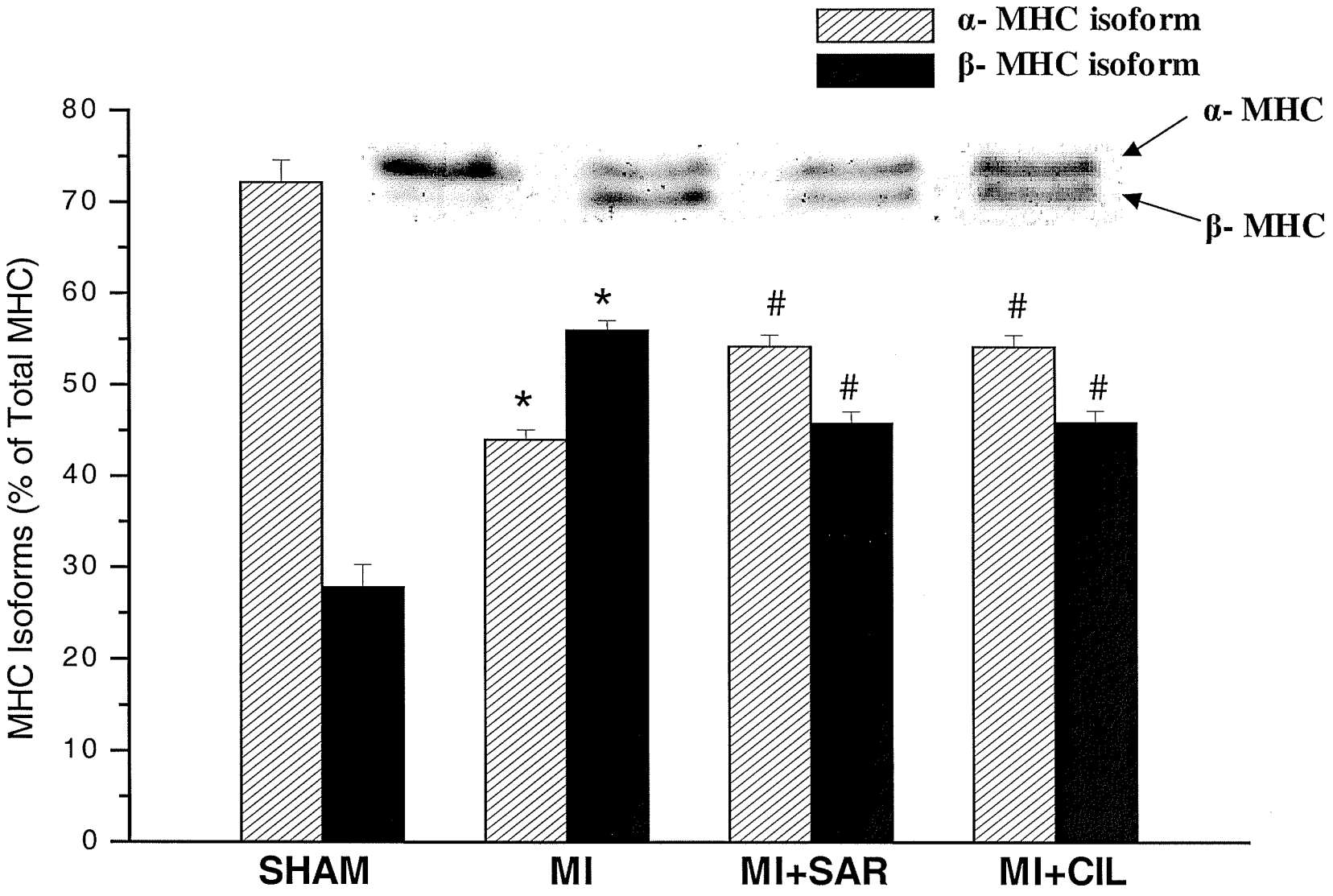

Figure 7. α- and β- MHC isoforms of sham, MI, SAR-treated and CIL-treated groups

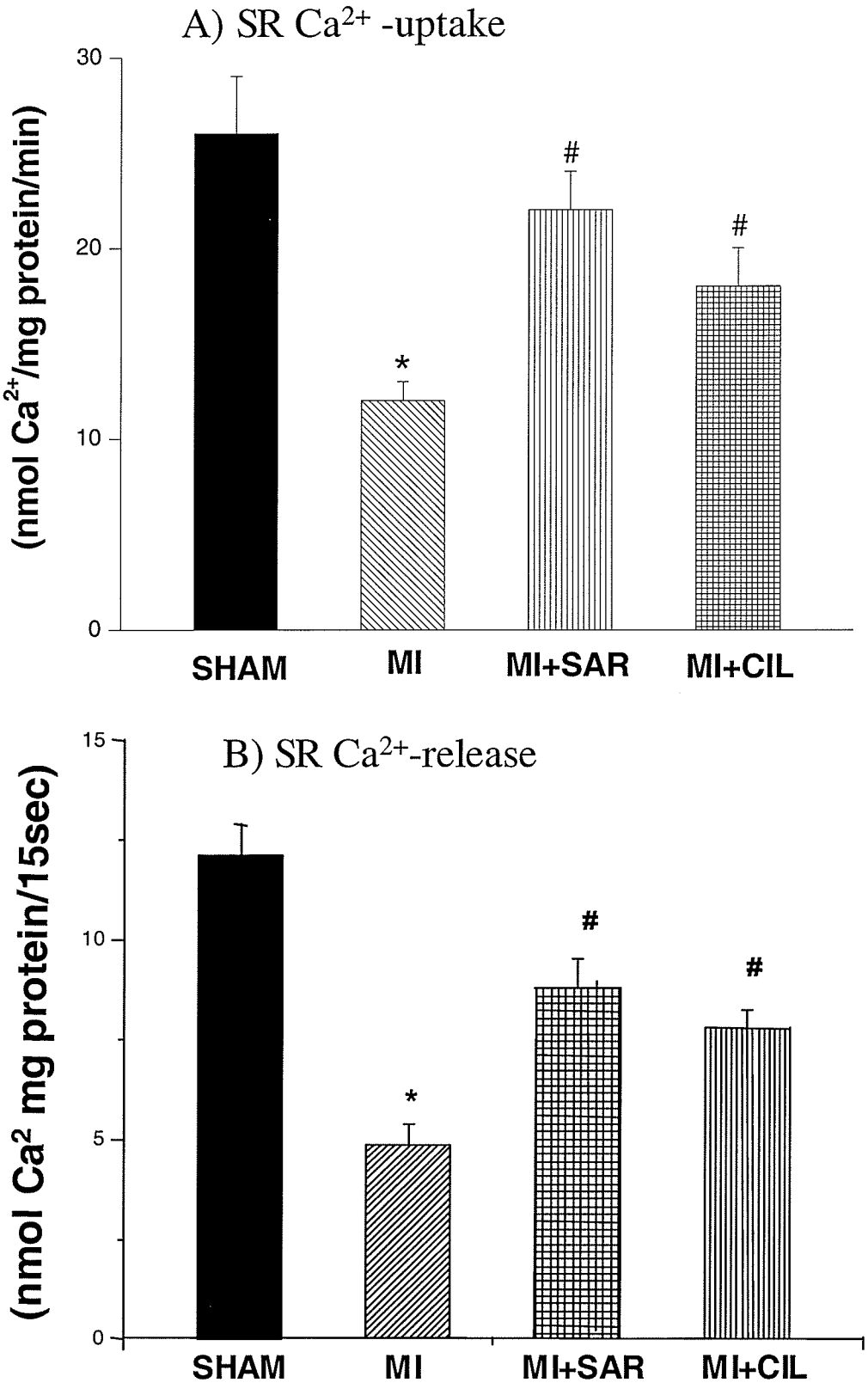

Figure 8. SR Ca^{2+}-uptake and Ca^{2+}-release activitiesof sham, MI, SAR-treated and CIL-treated groups

I. Effects of Sarpogrelate and Cilostazol on Gene Expression for Myofibrillar and SR Ca^{2+}-handling Proteins

Northern blot analysis of α-MHC, β-MHC, PLB, SERCA2a and RYR mRNA levels are shown in Figures 11 and 12. The α-MHC mRNA level was decreased by 70 % and that for β-MHC mRNA was increased by 88 % in LV of the infracted rats. These changes were significantly reversed by treatment with SAR and CIL. mRNA levels for SERCA2a, RyR and PLB proteins were decreased by 60 %, 45 % and 35 %, respectively. This decrease in LV mRNA levels for the SERCA2 and RyR was prevented by both SAR and CIL treatment. However, SAR and CIL failed to reverse the decreased mRNA levels of PLB.

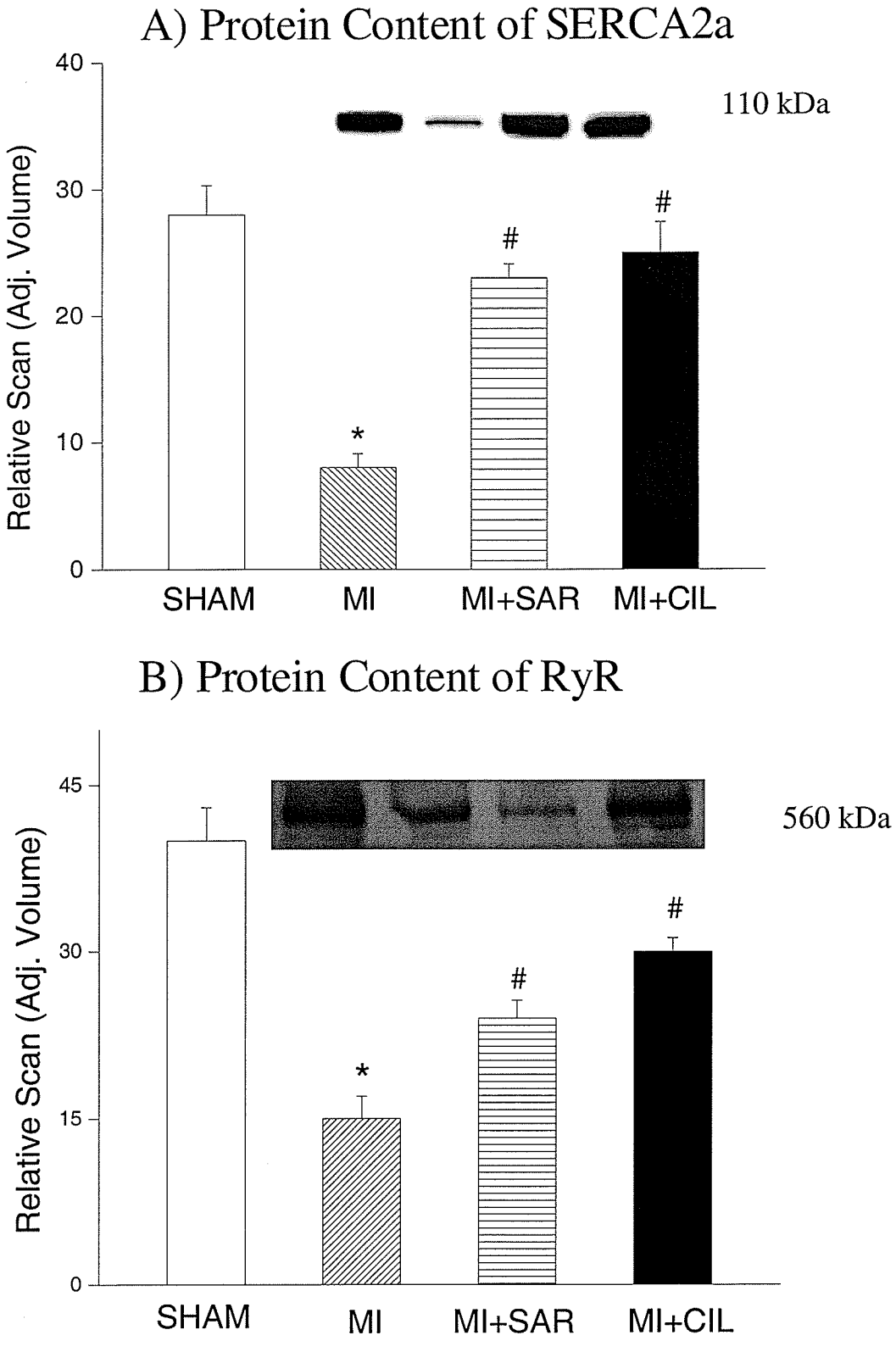

Figure 9. SR protein content of SERCA2a and RyR of sham, MI, SAR-treated and CIL-treated groups

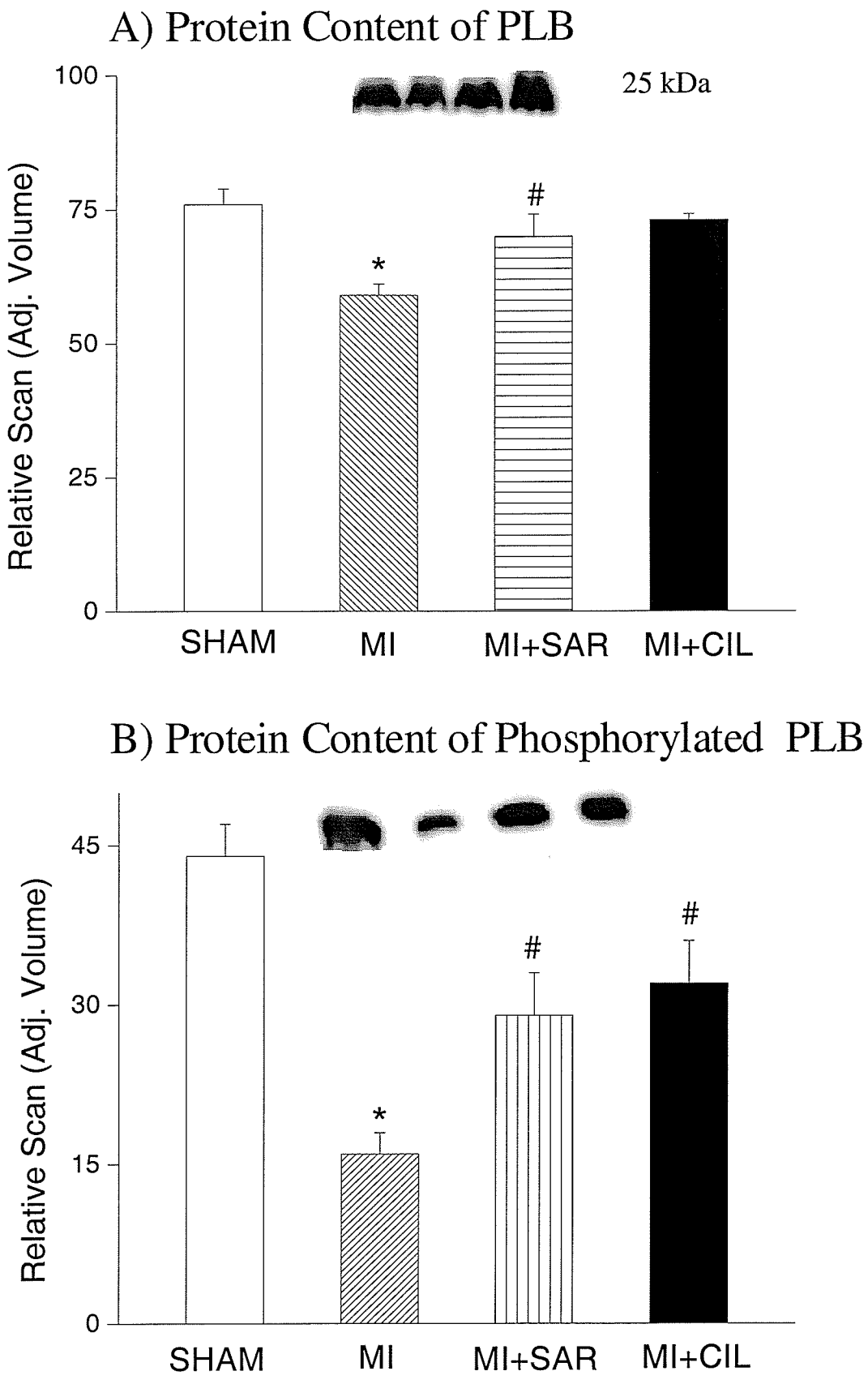

Figure 10. SR protein content of PLB and phosphorylated PLB of sham, MI, SAR-treatedand CIL-treated groups

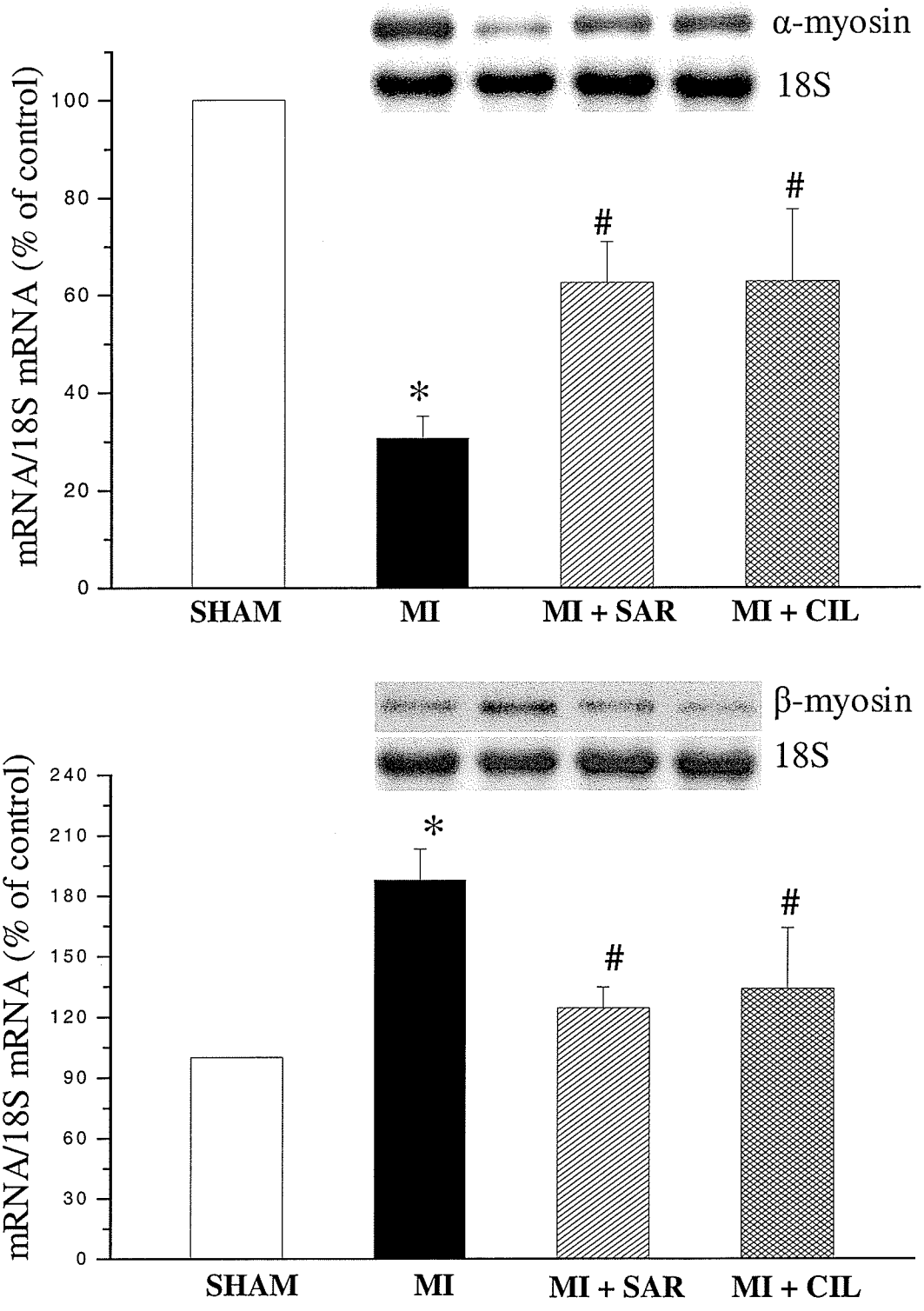

Figure 11. Northern blot analysis for α –MHC and β –MHC of sham, MI, SAR-treated and CIL-treated groups

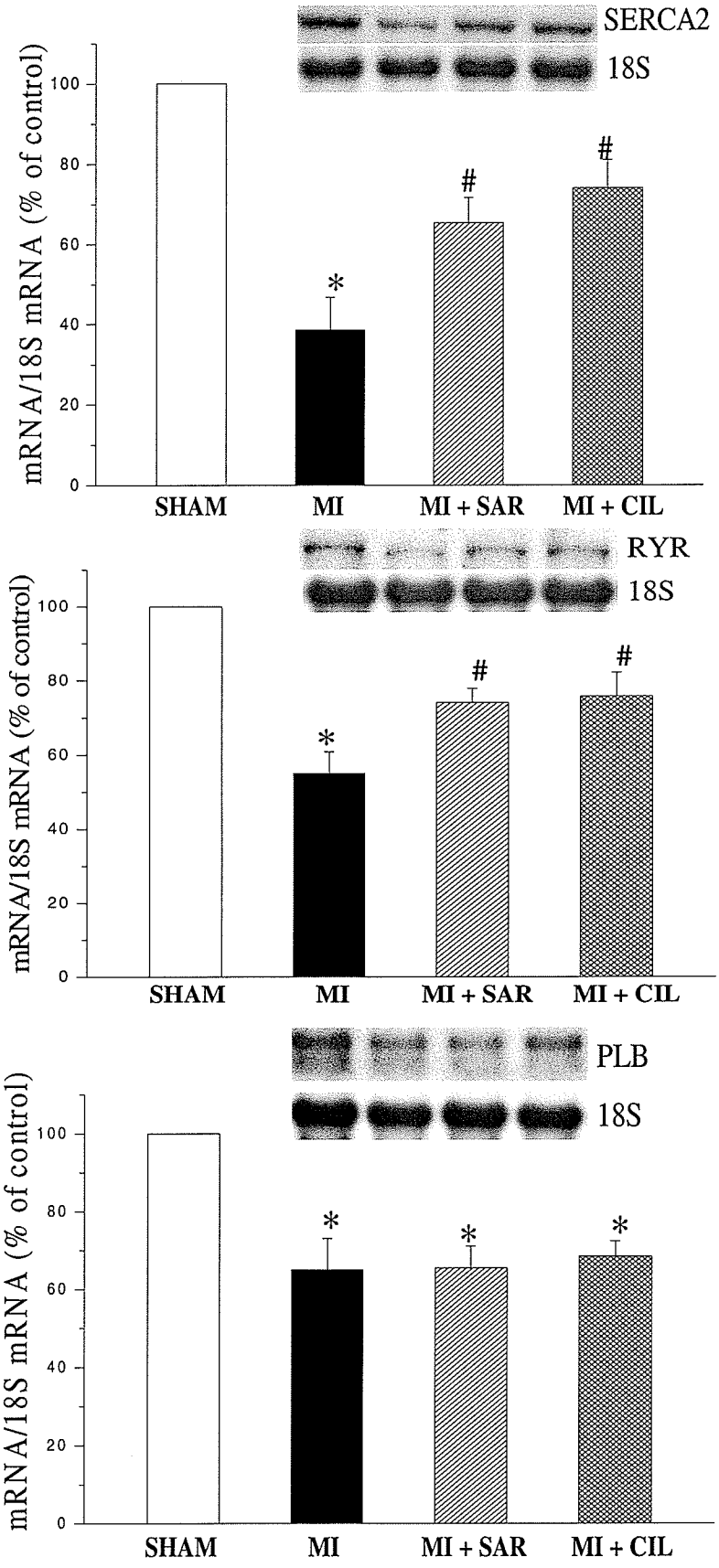

Figure 12. Northern blot analysis for SERCA2a, RyR and PLB of sham, MI, SAR-treated and CIL-treated groups

V. DISCUSSION

This study demonstrates for the first time that long-term (56 day) therapy with antiplatelet agents SAR and CIL significantly improved cardiac function and attenuated detrimental structural and subcellular remodeling in CHF in rats with large MI. Specifically, in comparison to untreated MI rats, treatment of MI rats with SAR or CIL impeded the development of chronic HF (manifested by reduced lung wt to body wtt ratio), preserved LV function and dimensions, reduced LV and RV hypertrophy, increased LVSP, MAP, ± dP/dt and decreased LVEDP in the infarcted animals. Both SAR and CIL prevented the depression of myofibrillar Ca^{2+}-stimulated ATPase activity, alteration in α-MHC and β-MHC isoforms and MHC gene expression in MI induced HF. In addition, treatment with these agents attenuated depressions in SR Ca^{2+} uptake and Ca^{2+} release, SR protein content for SERCA2a, RyR and PLB and SR gene expression for SERCA2a and RyR in the failing hearts. Together, these mechanisms may contribute to the preservation of LV structure and function observed with SAR and CIL treatment after MI in rats. Furthermore, SAR significantly prevented the mortality but CIL increased mortality in this model of HF. The mechanism of this increased survival in SAR needs further elucidation.

To our knowledge, no data are available on the long-term effects of antiplatelet agents on cardiac function, structural and subcellular remodeling in CHF. The CHF model in rats by coronary occlusion is well characterized and the pattern of evolution of overt HF is similar to humans in many features. This model has provided lot of information about the progression of LV dysfunction to HF. After a week of coronary occlusion, rats show signs of HF as evidenced by decrease in systolic blood pressure, increase in heart rate,

LVEDP and LV end-systolic and end-distolic diameters (13,215,328) followed by LV dilation, which occurs mostly during the first 4 weeks. These changes in LV leads to impaired systolic function as evidenced by progressive decrease in LVFS and LV posterior wall function. CO and SV were found to be significantly reduced in untreated CHF rats.

SAR and CIL treatments in infarcted animals reduced cardiac preload, as illustrated by the reduction of LVEDP. The decrease in blood pressure might also contribute, through decreased oxygen demand, to the beneficial effect of both SAR and CIL in CHF. The increase in lung wt and pulmonary edema in this experimental model may be due to diastolic dysfunction that was favorably modified by both SAR and CIL treatment. Indeed, both the drugs decreased LVEDP and increased rate of pressure development (+ dP/dt) and rate of pressure decay (- dP/dt) without having any significant effect on heart rate. There may be several possible explanations for the improvement in ventricular function by SAR and CIL treatments. Both the drugs tended to lower MAP and LVEDP, suggesting a trend toward a reduction of LV preload and afterload. The changes in the ventricular loading condition by SAR and CIL might improve LV function. Furthermore, decreases in LV load condition might have a favorable effect on cardiac energy metabolism. In this hemodynamic context, SAR and CIL treatments limited the increase in LV cavity enlargement in the failing hearts.

LVFS and LVEF were significantly improved by SAR and CIL treatments. This, together with the hemodynamic effects, as well as the decrease in LV intrinsic diameters and LV dilatation, may induce an improvement of LV filling and an increase in CO and cardiac contractility. This is confirmed in our study, in which SAR and CIL increased CO as well

as SV and prevented the deterioration of global LV function, with significant improvement in LV ± dP/dt. The lung wet wt/dry wt ratio of the rats with CHF was attenuated by treatment with SAR or CIL. This indicates that the drugs were effective in improving pulmonary edema in animals with CHF.

A. Effects of antiplatelet agents on myofibrillar remodeling in CHF

In the present study we have shown that a 5-week treatment with antiplatelet agents, SAR and CIL was found to improve cardiac dysfunction, attenuate the depression of myofibrillar Ca^{2+}-stimulated ATPase activity, alteration in α-MHC and β-MHC isoforms, and MHC gene expression in the LV from the infarcted rats.

The present study and previous reports from our laboratory have shown depressed cardiac function and myofibrillar ATPase activity as well as changes in MHC protein and gene expression in post-MI model of CHF (329,330). The observed decrease in myofibrillar Ca^{2+}-stimulated ATPase activity in failing hearts results in depression of cardiac function since the magnitude of cardiac contractile force is linearly related to myofibrillar Ca^{2+}-stimulated ATPase activity (331). On the other hand, myofibrillar ATPase activity is vastly determined by the ratio of the expressed MHC isoforms. α-MHC has a low ATPase activity but produces high cross-bridge force with more economy of energy consumption (332). At the molecular level, the consequence of CHF was studied in failing LV myocardium of explanted human hearts as well as in dogs and it was shown that mRNA gene expression of α-MHC was significantly reduced and β-MHC was significantly increased compared to normal (333). In rodent models of cardiac hypertrophy and failure, coordinate decrease in α-MHC and increase in β-MHC mRNA

and protein expression were found to be associated with a reduction in velocity of shortening and other measures of systolic function (334,335,336). This subserves an adaptation to the altered demand. Antiplatelet treatment with SAR and CIL was beneficial as evidenced by an increase in myofibrillar Ca^{2+}-stimulated ATPase activity, reversal of α-MHC and β-MHC isoform changes, increase in α-MHC mRNA, decrease in β-MHC mRNA and de-induction of the fetal gene program. The changes in MHC gene expression were translated into changes in protein expression, which potentially can explain the improved intrinsic systolic and diastolic function.

B. Effects of antiplatelet agents on sarcoplasmic reticular remodeling in CHF

In this study, most of the coronary ligated rats had approximately 38% infarction of the LV, 8 weeks after the operation. Afzal and Dhalla (152) have shown that the Ca^{2+} uptake activity of cardiac SR isolated from the failing rat heart was significantly decreased when compared to normal hearts. Our findings are compatible with the latter results. Treatment with antiplatelet agents reversed the decreased rate of SR Ca^{2+} uptake. Since a decrease in SR Ca^{2+} uptake plays a significant role in Ca^{2+} handling in the calcium induced calcium release mechanism (337), our results suggest that chronic therapy with antiplatelet agents, SAR and CIL is capable of improving the impaired Ca^{2+} handling ability of cardiac SR in CHF.

The ability of cardiac SR Ca^{2+} release was also examined in this study. It has been shown previously that the ability of SR to release Ca^{2+} is reduced in CHF. Ca^{2+} release mechanism plays a key role by which cytoplasmic Ca^{2+} concentration is elevated during cardiac contraction (338); the decrease in contraction in failing hearts may be attributed

to a defect in the ability to release Ca^{2+} from SR. Treatment with antiplatelet agents significantly reversed this effect, which may contribute to the recovery of cardiac contractile dysfunction in CHF.

The SR plays a significant role in the regulation of cytosolic calcium during EC coupling. SR stores Ca^{2+} and releases it into the cytosol to activate the contractile apparatus and Ca^{2+} reaccumulates in SR, subsequently. The ability of SR Ca^{2+} reuptake rate is mainly dependent on SERCA2a pump activity. In failing hearts, reduced amount of SERCA2a has been suggested as a mechanism for reduced SR calcium reuptake (339,310). SERCA2a mRNA level has been reported to be reduced both in the failing human (308,340,307) and rat myocardium (153). But there are diverging ideas about protein levels of both PLB and SERCA2a. Investigators claim that these proteins are unchanged in CHF (307, 342, 343, 344). It is reported that decreased SR Ca^{2+} uptake may occur by a reduction in SERCA2a pump activity without any change in the amount of SERCA2a or PLB. The phosphorylated PLB dissociates from SERCA2a leading to increase in SERCA2a pump activity but the unphosphorylated form inhibits SERCA2a pump activity. PLB is phosphorylated at 2 sites: serine^{16} via β-adrenergic pathway and threonine^{17} via calcium/calmodulin kinase II (345,346). Protein phosphatases (PP) are also known to regulate the degree of PLB phosphorylation: PP1 and PP2A are known to dephosphorylate PLB (347).

LV relaxation is closely related to Ca^{2+} uptake activity namely SERCA2a activity and Ca^{2+} uptake activities are enhanced when PLB is phosphorylated by cAMP dependent protein kinase (348). In human failing hearts, serine^{16} phosphorylated PLB is shown to be downregulated (343). Furthermore, Schwinger et al. (349), reported decreased levels of

both serine[16] and threonine[17] phosphorylation of PLB. The finding in our study that serine[16] phosphorylated PLB was significantly reduced, indicates a reduction in PKA-dependent PLB phosphorylation in CHF. This reduction in PKA-mediated PLB phosphorylation in CHF may be attributed to changes at various levels of β-adrenoceptor signaling pathways (350,351). The reduction in serine[16] phosphorylated PLB in CHF was attenuated by treatment with SAR and CIL.

The change in SERCA2a activity after decreasing phosphorylation in CHF suggests that the reduction in serine[16] phosphorylated PLB is one important factor determining the observed reduction in SR Ca^{2+} reuptake in MI. Previous studies have examined the levels of mRNA and protein for SERCA2a and PLB in various animal models and human HF. The results are controversial. The mRNA levels of PLB and SERCA2a have been reported to be reduced in failing human (352,308,353) and rat (152) hearts. With respect to the protein levels of PLB and SERCA2a there are diverging ideas (310, 342, 354, 355,307). Some investigators also indicated that in the majority of animal models of CHF there would be a reduced RNA and protein levels of SERCA2a and PLB (305).

C. Sarpogrelate as an antiplatlet agent in CHF

SAR is a novel antiplatelet agent used in Raynaud's phenomenon (356), Buerger's disease (357) and to prevent restenosis of coronary vessels after stent implantation (358). Platelets store serotonin and when platelets are disrupted, serotonin is released. The released serotonin can act on the $5HT_{2A}$ receptors of platelets to induce platelet aggregation and on VSM to cause vasoconstriction and both of these responses are responsible for thrombosis. Thus SAR has antithrombotic activity. Plasma 5HT levels

are increased in patients with coronary artery disease after angioplasty and stenting (359). Blockade of $5HT_2$ receptors by ketanserin and cinanserine can protect the isolated rat heart against ischemia (343). It is also shown that Cinanserine has a protective effect on pacing-induced HF in dogs (344). However, the role of serotonin and $5HT_2$ receptor blockers in myocardial ischemic cellular damage is unclear. The protype $5HT_{2A}$ antagonist, ketanserin, has shown beneficial clinical effects in CHF when given orally and intravenously. The drug improved mean CO, MAP, pulmonary capillary wedge and right atrial pressures, and systemic vascular resistance, at rest and with exercise (360,361,362,363). Ketanserin has also shown to cause regression of LV hypertrophy with preservation of systolic contractile function in hypertensive patients (364,365). Thus, further experiments are required to elucidate the mechanisms underlying the beneficial effects of SAR. Though the signaling mechanism is not clear for the beneficial effects of $5HT_{2A}$ receptor antagonists in CHF, Nebigil et al. (366), suggested that $5HT_{(2A,\ B,\ C)}$ receptors activates phospholipase C, which initiates a rapid release of inositol triphosphate and increases intracellular Ca^{2+} levels.

D. Cilostazol as an antiplatelet agent in CHF

The use of PDEIII inhibitors, which enhances the cyclic adenosine mono phosphate (cAMP) levels, has been viewed as a rational approach for the treatment of CHF, since the production of cAMP is deficient in terminal stages of CHF (367,368). Despite this appeal, there is considerable uncertainty about the long-term efficacy and safety of these drugs in patients with CHF (369,370). It has been postulated by various investigators that uncontrolled and controlled studies with cAMP PDEIII inhibitors may

accelerate the progression of CHF and provoke serious ventricular arrhythmias (370,371,372). PDEIII inhibitors like amrinone, milrinone and vesnarinone have been studied in HF. Milrinone is a potent cardiac bipyridine with inotropic and vasodilator properties (373,374), which has been used to treat patients with CHF. It is known to exhibit beneficial inotropic effect by inhibiting the breakdown of cAMP and, hence, elevating the cellular cAMP (374,375). The cellular cAMP activates cAMP-dependent protein kinases, which results in increase in influx of Ca^{2+} (14) and the rate of Ca^{2+} uptake by the SR (376).

The inhibition of phosphodiesterase activity by PDEIII inhibitors increases the concentration of cAMP in the cell. There are several mechanisms by which increased levels of (377-380) cAMP may augment myocardial contractility:

(a) phosphoralative modulation of functional cellular proteins increases Ca^{2+} influx through the Ca^{2+} channels, (b) the cytoplasmic Ca^{2+} levels available to troponin is increased by inhibiting the Ca^{2+} uptake by SR, and (c) by blocking receptor for endogenous negative inotropic mediator, adenosine. CIL is a newly developed antiplatelet agent with PDEIII inhibitory effect for the management of intermittent caludication and peripheral vascular disease. CIL inhibits platelet aggregation and promotes vasodilation, an effect that is mediated by PGE1 and also in vitro via PDEIII inhibition and subsequent cAMP accumulation in blood platelets and blood vessels (381). Milrinone and amrinone exert positive inotropic effects and peripheral vasodilatory effects and thus improving symptoms of CHF but these drugs are not used in treating intermittent claudication. The reasons for the differences between the pharmacological actions of various PDEIII inhibitors are not known. Antiplatelet drug treatment with CIL

is also shown to prevent restenosis of coronary vessels after stent implantation (382). CREST (Cilostazol for RESTenosis) is an ongoing randomized clinical trial to test the effects of CIL to prevent restenosis following uncomplicated stent implantation.

Our findings indicate that long-term treatment with CIL has a deleterious effect on the survival of rats with CHF. The results of the present trial are consistent with the findings of previous clinical studies carried out with cAMP PDEIII inhibitors like milrinone (371) and enoximone (383) in patients with CHF.

Experimental results in rat model of CHF showed contrasting results: milrinone improved cardiac performance and survival (384,385), attenuated ventricular remodeling to the extent similar to that achieved with ACE inhibitors. To address these discordant findings of experimental and clinical studies with phosphodiesterase inhibitors, we conducted this study with CIL on CHF induced by MI.

E. Electrocardiographic changes and treatment with SAR and CIL

While ST segment and T wave changes are usually transitory in MI and recover with time, pathological Q waves or QS complexes representing myocardial necrosis and scar formation persist. The relationship between infarct size and Q wave is directly proportional, the greater the depth of the infarct, the deeper the Q waves. A deep QS complex results in complete disappearance of R wave in large infarctions. After MI, remodeling process starts in the remaining working myocardium as well as in the infracted zone (13). Although the scar cannot be replaced by working myocardial cells, ECG changes might occur. In our experimental conditions, treatment with SAR or CIL induced no marked changes in QRS morphology. PQ interval was found prolonged in

the infarcted animals probably due to compromised AV conduction caused by the extensive MI. Coronary artery occlusion has been reported to produce a marked prolongation of the QT_c interval (386), which occurs in the acute phase of MI, slowly recovers in the following weeks but it does not necessarily return to normal. QT_c prolongation is of functional importance because it predisposes the heart for malignant arrhythmias (387). At 8 weeks after MI, prolongation of QT_c interval was still persisting in untreated MI rats when compared to control animals, which was not altered by either SAR or CIL treatment. In this study we did not focus on the rate of arrhythmias because it would have required longer ECG recordings.

The precise mechanism by which long-term treatment with other PDEIII inhibitors like milrinone that increases mortality in patients with CHF remains elucidation. It has been speculated that, drug induced increases in intracellular cAMP may have directly toxic effects on myocardial cells which can lead to rhythm disturbances (388,389). Despite its favorable effects on hemodynamics and ventricular remodeling, long-term therapy with CIL increases mortality in rats. Scholz and Meyer (390) postulated that cAMP could be arrhythmogenic by several different mechanisms: (a) secondary to cAMP increased rates of diastolic depolarization could lead to abnormal impulse generation, (b) secondary to triggered activity from early or delayed after depolarizations or from provocation of slow responses in depolarized tissues can also lead to abnormal impulses and third, frequency of arrhythmias could be increased by inotropic agents which increase myocardial oxygen consumption.

VI. CONCLUSIONS

This study was undertaken to test the beneficial effects of antiplatelet agents, SAR or CIL, on LV function and subcellular remodeling in post-MI CHF. From the results obtained in this study, the following conclusions can be drawn-

1) CHF in rats is evidenced by decrease in systolic blood pressure, increase in heart rate, LVEDP and LV end-systolic and end-diastolic diameters followed by LV dilation. These changes in LV leads to impaired systolic and diastolic function and were related to depressed myofibrillar ATPase activity as well as decreased MHC protein and gene expression.
2) In CHF, alterations in SR Ca^{2+} uptake and Ca^{2+} release activities as well as changes in SR protein content and gene expression for SERCA2a and PLB are related to LV dysfunction and abnormalities in Ca^{2+} handling by cardiomyocytes. The change in SERCA2a activity and decreased SR phosphorylation in CHF suggests that the reduction in serine[16] phosphorylated PLB is one important factor determining the observed reduction in SR Ca^{2+} reuptake in MI.
3) In view of observed alterations in myofibrillar and SR gene expression and protein contents, it is suggested that ventricular remodeling, systolic and diastolic dysfunction in CHF, may be partly due to the subcellular remodeling of the myofibrils and SR.
4) Treatment of infarcted animals with antiplatelet agents, SAR and CIL significantly improved the LV function and reversed the subcellular remodeling in myofibrils and SR due to post-MI CHF. Such changes may be indirectly due to decreas in preload and afterload.

5) Though SAR and CIL had beneficial effects to the same extent on overall cardiac function; however, CIL was observed to increase mortality, which may be attributed to VT.

VII. REFERENCES

1. McFate SW. Epidemiology of congestive heart failure. Am J Cardiol 1985;55 (Suppl A):3–8.
2. Paul SD, Kuntz KM, Eagle KA, Weinstein MC. Cost and effectiveness of angiotensin converting enzyme inhibition in patients with congestive heart failure. Arch Intern Med 1994;154:1143–1149.
3. Bristow MR. New approaches to therapy for congestive heart failure. American Heart Association Twenty-First Science Writers Forum, Clearwater, FL, 1994.
4. Mann DL, Taegtmeyer H. Dynamic regulation of the extracellular matrix after mechanical unloading of the failing human heart: recovering the missing link in left ventricular remodeling. Circulation 2001;104:1089–1091.
5. Mann DL. Mechanisms and models in heart failure: A combinatorial approach. Circulation 1999;100:999–1008.
6. Sharpe N. Left ventricular remodeling: pathophysiology and treatment. Heart Fail Monit 2003;4:55-61.
7. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation. 1986;74:1124-1136.
8. Habib GB, Heibig J, Forman SA, Brown BG, Roberts R, Terrin ML, Bolli R. Influence of coronary collateral vessels on myocardial infarct size in humans. Results of phase I thrombolysis in myocardial infarction (TIMI) trial. The TIMI Investigators. Circulation 1991;83:739-746.
9. The ISIS-2 Collaborative Group. Second international study of infarct survival. Lancet 1988;2:349-360.

10. Braunwald E, Pfeffer MA. Ventricular enlargement and remodeling following acute myocardial infarction: mechanisms and management. Am J Cardiol 1991;68:1D-6D.

11. White HD, Norris RM, Brown MA, Brandt PW, Whitlock RM, Wild CJ. Left ventricular end-systolic volume as the major determinant of survival after recovery from myocardial infarction. Circulation 1987;76:44-51.

12. Patten RD, Udelson JE, Konstam MA. Ventricular remodeling and its prevention in the treatment of heart failure. Curr Opin Cardiol 1998;13:162-167.

13. Pfeffer MA, Braunwald E. Ventricular remodeling after myocardial infarction. Experimental observations and clinical implications. Circulation 1990;81:1161-1172.

14. Olivetti G, Capasso JM, Sonnenblick EH, Anversa P. Side-to-side slippage of myocytes participates in ventricular wall remodeling acutely after myocardial infarction in rats. Circ Res 1990;67:23-34.

15. Weisman HF, Bush DE, Mannisi JA, Weisfeldt ML, Healy B. Cellular mechanisms of myocardial infarct expansion. Circulation 1988;78:186-201.

16. Christe ME, Rodgers RL. Altered glucose and fatty acid oxidation in hearts of the spontaneously hypertensive rat. J Mol Cell Cardiol 1994;26:1371-1375.

17. Cleutjens JP, Kandala JC, Guarda E, Guntaka RV, Weber KT. Regulation of collagen degradation in the rat myocardium after infarction. J Mol Cell Cardiol 1995;27:1281-1292.

18. Warren SE, Royal HD, Markis JE, Grossman W, McKay RG. Time course of left ventricular dilation after myocardial infarction: influence of infarct-related artery

and success of coronary thrombolysis. J Am Coll Cardiol 1988;11:12-19.

19. Rumberger JA, Behrenbeck T, Breen JR, Reed JE, Gersh BJ. Nonparallel changes in global left ventricular chamber volume and muscle mass during the first year after transmural myocardial infarction in humans. J Am Coll Cardiol 1993;21:673-682.

20. Beltrami CA, Finato N, Rocco M, Feruglio GA, Puricelli C, Cigola E, Quaini F, Sonnenblick EH, Olivetti G, Anversa P. Structural basis of end-stage failure in ischemic cardiomyopathy in humans. Circulation 1994;89:151-163.

21. Lew WY, Chen ZY, Guth B, Covell JW. Mechanisms of augmented segment shortening in nonischemic areas during acute ischemia of the canine left ventricle. Circ Res 1985;56:351-358.

22. Gerdes AM, Kellerman SE, Moore JA, Muffly KE, Clark LC, Reaves PY, Malec KB, McKeown PP, Schocken DD. Structural remodeling of cardiac myocytes in patients with ischemic cardiomyopathy. Circulation 1992;86:426-430.

23. Anversa P, Beghi C, Kikkawa Y, Olivetti G. Myocardial response to infarction in the rat. Morphometric measurement of infarct size and myocyte cellular hypertrophy. Am J Pathol 1985;118:484-492.

24. Erlebacher JA, Weiss JL, Weisfeldt ML, Bulkley BH. Early dilation of the infarcted segment in acute transmural myocardial infarction: role of infarct expansion in acute left ventricular enlargement. J Am Coll Cardiol 1984;4:201-208.

25. Mitchell GF, Lamas GA, Vaughan DE, Pfeffer MA. Left ventricular remodeling in the year after first anterior myocardial infarction: a quantitative analysis of

contractile segment lengths and ventricular shape. J Am Coll Cardiol 1992;19:1136-1144.

26. Stillwell GK. The Law of Laplace. Some clinical applications. Mayo Clin Proc 1973;48:863-869.

27. Pfeffer JM, Pfeffer MA, Fletcher PJ, Braunwald E. Progressive ventricular remodeling in rat with myocardial infarction. Am J Physiol Heart Circ 1991;260:H1406-H1414.

28. Benedict CR, Francis GS, Shelton B, Johnstone DE, Kubo SH, Kirlin P, Nicklas J, Liang CS, Konstam MA, Greenberg B, Yusuf S. Effect of long-term enalapril therapy on neurohormones in patients with left ventricular dysfunction. SOLVD Investigators. Am J Cardiol 1995;75:1151-1157.

29. Kono T, Sabbah HN, Rosman H, Alam M, Jafri S, Goldstein S. Left ventricular shape is the primary determinant of functional mitral regurgitation in heart failure. J Am Coll Cardiol 1992;20:1594-1598.

30. Mann DL. Mechanisms and models in heart failure: A combinatorial approach. Circulation 1999;100:999-1008.

31. Kanekar S, Hirozanne T, Terracio L, Borg TK. Cardiac fibroblasts: Form and function. Cardiovascular Pathology 1998;7:127–133.

32. Weber KT, Sun Y, Tyagi SC, Cleutjens JP. Collagen network of the myocardium: function, structural remodeling and regulatory mechanisms. J Mol Cell Cardiol 1994;26:279-292.

33. Takano H, Hasegawa H, Nagai T, Komuro I. Implication of cardiac remodeling in heart failure: mechanisms and therapeutic strategies. Intern Med 2003;42:465-

469.

34. Hutchins GM, Bulkley BH. Infarct expansion versus extension: two different complications of acute myocardial infarction. Am J Cardiol 1978;41:1127-1132.

35. Weisman HF, Bush DE, Mannisi JA, Weisfeldt ML, Healy B. Cellular mechanisms of myocardial infarct expansion. Circulation 1988;78:186-201.

36. Zhao MJ, Zhang H, Robinson TF, Factor SM, Sonnenblick EH, Eng C. Profound structural alterations of the extracellular collagen matrix in postischemic dysfunctional ("stunned") but viable myocardium. J Am Coll Cardiol 1987;10:1322-1334.

37. Sato S, Ashraf M, Millard RW, Fujiwara H, Schwartz A. Connective tissue changes in early ischemia of porcine myocardium: an ultrastructural study. J Mol Cell Cardiol 1983;15:261-275.

38. Vracko R, Thorning D, Frederickson RG. Connective tissue cells in healing rat myocardium. A study of cell reactions in rhythmically contracting environment. Am J Pathol 1989;134:993-1006.

39. Bouchardy B, Majno G. Histopathology of early myocardial infarcts. A new approach. Am J Pathol 1974;74:301-330.

40. Kramer CM, Lima JA, Reichek N, Ferrari VA, Llaneras MR, Palmon LC, Yeh IT, Tallant B, Axel L. Regional differences in function within noninfarcted myocardium during left ventricular remodeling. Circulation 1993;88:1279-1288.

41. Eaton LW, Weiss JL, Bulkley BH, Garrison JB, Weisfeldt ML. Regional cardiac dilatation after acute myocardial infarction: recognition by two-dimensional echocardiography. N Engl J Med 1979;300:57-62.

42. Erlebacher JA, Weiss JL, Eaton LW, Kallman C, Weisfeldt ML, Bulkley BH. Late effects of acute infarct dilation on heart size: a two dimensional echocardiographic study. Am J Cardiol 1982;49:1120-1126.

43. Schuster EH, Bulkley BH. Expansion of transmural myocardial infarction: a pathophysiologic factor in cardiac rupture. Circulation 1979;60:1532-1538.

44. Jugdutt BI, Michorowski BL. Role of infarct expansion in rupture of the ventricular septum after acute myocardial infarction: a two-dimensional echocardiographic study. Clin Cardiol 1987;10:641-652.

45. Pirolo JS, Hutchins GM, Moore GW. Infarct expansion: pathologic analysis of 204 patients with a single myocardial infarct. J Am Coll Cardiol 1986;7:349-354.

46. Narula J, Haider N, Virmani R, DiSalvo TG, Kolodgie FD, Hajjar RJ, Schmidt U, Semigran MJ, Dec GW, Khaw BA. Apoptosis in myocytes in end-stage heart failure. N Engl J Med 1996;335:1182-1189.

47. Olivetti G, Abbi R, Quaini F, Kajstura J, Cheng W, Nitahara JA, Quani E, Di Loreto C, Beltrami CA, Krajewski S, Reed JC, Anversa P. Apoptosis in the failing human heart. N Engl J Med 1997;336:1131-1141.

48. Communal C, Singh K, Pimentel DR, Colucci WS. Norepinephrine stimulates apoptosis in adult rat ventricular myocytes by activation of the beta-adrenergic pathway. Circulation 1998;98:1329-1334.

49. Leri A, Claudio PP, Li Q, Wang X, Reiss K, Wang S, Malhotra A, Kajstura J, Anversa P. Stretch-mediated release of angiotensin II induces myocyte apoptosis by activating p53 that enhances the local renin-angiotensin system and decreases the Bcl-2-to-Bax protein ratio in the cell. J Clin Invest 1998;101:1326-1342.

50. Krown KA, Page MT, Nguyen C, Zechner D, Gutierrez V, Comstock KL, Glembotski CC, Quintana PJ, Sabbadini RA. Tumor necrosis factor alpha-induced apoptosis in cardiac myocytes. Involvement of the sphingolipid signaling cascade in cardiac cell death. J Clin Invest 1996;98:2854-2865.

51. Spinale FG. Matrix metalloproteinases: regulation and dysregulation in the failing heart. Circ Res 2002;90:520-530.

52. Hirohata S, Kusachi S, Murakami M, Murakami T, Sano I, Watanabe T, Komatsubara I, Kondo J, Tsuji T. Time dependent alterations of serum matrix metalloproteinase-1 and metalloproteinase-1 tissue inhibitor after successful reperfusion of acute myocardial infarction. Heart 1997;78:278-284.

53. Siwik DA, Chang DL, Colucci WS. Interleukin-1beta and tumor necrosis factor-alpha decrease collagen synthesis and increase matrix metalloproteinase activity in cardiac fibroblasts in vitro. Circ Res 2000;86:1259-1265.

54. Siwik DA, Pagano PJ, Colucci WS. Oxidative stress regulates collagen synthesis and matrix metalloproteinase activity in cardiac fibroblasts. Am J Physiol Cell Physiol 2001;280:C53-C60.

55. Kim HE, Dalal SS, Young E, Legato MJ, Weisfeldt ML, D'Armiento J. Disruption of the myocardial extracellular matrix leads to cardiac dysfunction. J Clin Invest 2000;106:857-866.

56. Ducharme A, Frantz S, Aikawa M, Rabkin E, Lindsey M, Rohde LE, Schoen FJ, Kelly RA, Werb Z, Libby P, Lee RT. Targeted deletion of matrix metalloproteinase-9 attenuates left ventricular enlargement and collagen accumulation after experimental myocardial infarction. J Clin Invest

2000;106:55-62.

57. Roten L, Nemoto S, Simsic J, Coker ML, Rao V, Baicu S, Defreyte G, Soloway PJ, Zile MR, Spinale FG. Effects of gene deletion of the tissue inhibitor of the matrix metalloproteinase-type 1 (TIMP-1) on left ventricular geometry and function in mice. J Mol Cell Cardiol 2000;32:109-120.

58. Spinale FG, Coker ML, Krombach SR. Matrix metalloproteinase inhibition during the development of congestive heart failure. effects on left ventricular dimensions and function. Circ Res 1999;85:364-376.

59. Creemers EE, Cleutjens JP, Smits JF, Daemen MJ. Matrix metalloproteinase inhibition after myocardial infarction. A new approach to prevent heart failure? Circ Res 2001;89:201-210.

60. Levine B, Kalman J, Mayer L, Fillit HM,Packer M. Elevated circulating levels of tumor necrosis factor in severe chronic heart failure. N Engl J Med 1990;223:236–241.

61. Bradham WS, Bozkurt B, Gunasinghe H, Mann D, Spinale FG. Tumor necrosis factor-alpha and myocardial remodeling in progression of heart failure: a current perspective. Cardiovasc Res 2002;53:822-830.

62. Torre-Amione G, Kapadia S, Benedict C, Oral H, Young JB, Mann DL. Proinflammatory cytokine levels in patients with depressed left ventricular ejection fraction: a report from the Studies of Left Ventricular Dysfunction (SOLVD). J Am Coll Cardiol 1996;27:1201–1206.

63. Birks EJ, Burton PB, Owen V, Mullen AJ, Hunt D, Banner NR, Barton PJ, Yacoub MH. Elevated tumor necrosis factor-alpha and interleukin-6 in

myocardium and serum of malfunctioning donor hearts. Circulation 2000;102:352–358.

64. Torre-Amione G, Kapadia S, Lee J, Durand JB, Bies RD, Young JB, Mann DL. Tumor necrosis factor-α and tumor necrosis factor receptors in the failing human heart. Circulation 1996;93:704–711.

65. Habib FM, Springall DR, Davies GJ, Oakley CM, Yacoub MH, Polak JM. Tumour necrosis factor and inducible nitric oxide synthase in dilated cardiomyopathy. Lancet 1996;347:1151–1155.

66. Bozkurt B, Kribbs SB, Clubb FJ Jr, Michael LH, Didenko VV, Hornsby PJ, Seta Y, Oral H, Spinale FG, Mann DL. Pathophysiologically relevant concentrations of tumor necrosis factor-a promote progressive left ventricular dysfunction and remodeling in rats. Circulation 1998;97:1382–1391.

67. Li YY, Feng YQ, Kadokami T, McTiernan CF, Draviam R, Watkins SC, Feldman AM. Myocardial extracellular matrix remodeling in transgenic mice overexpressing tumor necrosis factor alpha can be modulated by anti-tumor necrosis factor alpha therapy. Proc Natl Acad Sci USA. 2000;97:12746-12751.

68. Ungureanu-Longrois D, Balligand JL, Simmons WW, Okada I, Kobzik L, Lowenstein CJ, Kunsel SL, Michel T, Kelly RA, Smith TW. Induction of nitric oxide synthase activity by cytokines in ventricular myocytes is necessary but not sufficient to decrease contractile responsiveness to β-adrenergic agonists. Circ Res 1995;77:494–502.

69. Li X, Moody MR, Engel D, Walker S, Clubb FJ Jr, Sivasubramanan N, Mann DL, Reid MB. Cardiac-specific overexpression of tumor necrosis factor-α causes

oxidative stress and contractile dysfunction in mouse diaphragm. Circulation 2000;102:1690–1696.

70. Remme WJ. Pharmacological modulation of cardiovascular remodeling: a guide to heart failure therapy. Cardiovasc Drugs Ther 2003;17:349-360.

71. Nakamura K, Fushimi K, Kouchi H, Mihara K, Miyazaki M, Ohe T, Namba M. Inhibitory effects of antioxidants on neonatal rat cardiac myocyte hypertrophy induced by tumor necrosis factor-α and angiotensin II. Circulation 1998;98:794–799.

72. Feuerstein GZ, Young PR. Apoptosis in cardiac diseases: Stress- and mitogen-activated signaling pathways. Cardiovasc Res 2000;45:560–569.

73. Wollert KC, Heineke J, Westermann J, Ludde M, Fiedler B, Zierhut W, Laurent D, Bauer MK, Schulze-Osthoff K, Drexler H. The cardiac Fas (APO-1/CD95) Receptor/Fas ligand system: relation to diastolic wall stress in volume-overload hypertrophy in vivo and activation of the transcription factor AP-1 in cardiac myocytes. Circulation 2000;101:1172–1178.

74. Gurantz D, Cowling RT, Villarreal FJ, Greenberg BH. Tumor necrosis factor-alpha upregulates angiotensin II type 1 receptors on cardiac fibroblasts. Circ Res 1999;85:272-279.

75. Schultz Jel J, Witt SA, Glascock BJ, Nieman ML, Reiser PJ, Nix SL, Kimball TR, Doetschman T. TGF-beta1 mediates the hypertrophic cardiomyocyte growth induced by angiotensin II. J Clin Invest 2002;109:787-796.

76. van Wamel AJ, Ruwhof C, van der Valk-Kokshoom LE, Schrier PI, van der Laarse A. The role of angiotensin II, endothelin-1 and transforming growth

factor-beta as autocrine/paracrine mediators of stretch-induced cardiomyocyte hypertrophy. Mol Cell Biochem 2001;218:113-1124.

77. Paul S. The pathophysiologic process of ventricular remodeling: from infarct to failure. Crit Care Nurs Q 1995;18:7-21.

78. Theroux P, Ross J Jr, Franklin D, Covell JW, Bloor CM, Sasayama S. Regional myocardial function and dimensions early and late after myocardial infarction in the unanesthetized dog. Circ Res 1977;40:158-165.

79. Rumberger JA, Behrenbeck T, Breen JR, Reed JE, Gersh BJ. Nonparallel changes in global left ventricular chamber volume and muscle mass during the first year after transmural myocardial infarction in humans. J Am Coll Cardiol 1993;21:673-682.

80. Olivetti G, Capasso JM, Meggs LG, Sonnenblick EH, Anversa P. Cellular basis of chronic ventricular remodeling after myocardial infarction in rats. Circ Res. 1991;68:856-869.

81. Grossman W. Cardiac hypertrophy: useful adaptation or pathologic process? Am J Med 1980;69:576-584.

82. Gerdes AM. Remodeling of ventricular myocytes during cardiac hypertrophy and heart failure. J Fla Med Assoc 1992;79:253-255.

83. Lamas GA, Pfeffer MA. Increased left ventricular volume following myocardial infarction in man. Am Heart J 1986;111:30-35.

84. Kitamura S, Kay JH, Krohn BG, Magidson O, Dunne EF. Geometric and functional abnormalities of the left ventricle with a chronic localized noncontractile area. Am J Cardiol 1973;31:701-707.

85. Hamilton GW, Murray JA, Kennedy JW. Quantitative angiocardioraphy in ischemic heart disease. The spectrum of abnormal left ventricular function and the role of abnormally contracting segments. Circulation 1972;45:1065-1086.

86. Feild BJ, Russell RO Jr, Dowling JT, Rackley CE. Regional left ventricular performance in the year following myocardial infarction. Circulation 1972;46:679-689.

87. Swan HJ, Forrester JS, Diamond G, Chatterjee K, Parmley WW. Hemodynamic spectrum of myocardial infarction and cardiogenic shock. A conceptual model. Circulation 1972;45:1097-1110.

88. Hori M, Inoue M, Mishima M, Shimazu T, Abe H, Fukui S, Ohgitani N, Minamino T. Infarct size and left ventricular ejection fraction in acute myocardial infarction. Jpn Circ J 1977;41:1280-1282 and 1299-1306.

89. Bonaduce D, Petretta M, Morgano G, Villari B, Bianchi V, Conforti G, Salemme L, Themistoclakis S, Pulcino A. Left ventricular remodeling in the year after myocardial infarction: an echocardiographic, haemodynamic, and radionuclide angiographic study. Coron Artery Dis 1994;5:155-162.

90. Katz AM. Cardiomyopathy of overload. A major determinant of prognosis in congestive heart failure. N Engl J Med 1990 11;322:100-110.

91. McAlpine HM, Morton JJ, Leckie B, Rumley A, Gillen G, Dargie HJ. Neuroendocrine activation after acute myocardial infarction. Br Heart J 1988;60:117-124.

92. Russell SD, DeWald T. Vasopressin receptor antagonists: therapeutic potential in the management of acute and chronic heart failure. Am J Cardiovasc Drugs

2003;3:13–20.

93. Yousef ZR, Redwood SR, Marber MS. Postinfarction left ventricular remodeling: a pathophysiological and therapeutic review. Cardiovasc Drugs Ther 2000;14:243-252.

94. Esler M, Jennings G, Korner P, Willett I, Dudley F, Hasking G, Anderson W, Lambert G. Assessment of human sympathetic nervous system activity from measurements of norepinephrine turnover. Hypertension 1988;11:3–20.

95. Sutton MG, Sharpe N. Left ventricular remodeling after myocardial infarction: pathophysiology and therapy. Circulation 2000;101:2981–2988.

96. Weber KT. Extracellular matrix remodeling in heart failure: a role for de novo angiotensin II generation. Circulation 1997;96:4065–4082.

97. Griendling KK, Sorescu D, Ushio-Fukai M. NAD(P)H oxidase: role in cardiovascular biology and disease. Circ Res 2000;86:494–501.

98. Dhalla AK, Hill MF, Singal PK. Role of oxidative stress in transition of hypertrophy to heart failure. J Am Coll Cardiol 1996;28:506–514.

99. Kinugawa S, Tsutsui H, Hayashidani S, Ide T, Suematsu N, Satoh S, Utsumi H, Takeshita A. Treatment with dimethylthiourea prevents left ventricular remodeling and failure after experimental myocardial infarction in mice: role of oxidative stress. Circ Res 2000;87:392–398.

100. Gyurko R, Kuhlencordt P, Fishman MC, Huang PL. Modulation of mouse cardiac function in vivo by Enos and ANP. Am J Physiol Heart Circ Physiol 2000;278: H971–H981.

101. Gruppo Italiano per lo Studio della Sopravvivenza nell'infarto Miocardico.

GISSI-3: effects of lisinopril and transdermal glyceryl trinitrate singly and together on 6-week mortality and ventricular function after acute myocardial infarction. Lancet 1994;343:1115–1122.

102. Ritchie RH, Schiebinger RJ, LaPointe MC, Marsh JD. Angiotensin II-induced hypertrophy of adult rat cardiomyocytes is blocked by nitric oxide. Am J Physiol 1998;275:H1370–H1374.

103. Murohara T, Asahara T, Silver M, Bauters C, Masuda H, Kalka C, Kearney M, Chen D, Chen D, Symes JF, Fishman MC, Huang PL, Isner JM. Nitric oxide synthase modulates angiogenesis in response to tissue ischemia. J Clin Invest 1998;101:2567–2578.

104. Kim NN, Villegas S, Summerour SR, Villarreal FJ. Regulation of cardiac fibroblast extracellular matrix production by bradykinin and nitric oxide. J Mol Cell Cardiol 1999;31:457–466.

105. Scherrer-Crosbie M, Ullrich R, Bloch KD, Nakajima H, Nasseri B, Aretz T, Lindsey ML, Vancon A, Huang PL, Lee RT, Zapol WM, Picard MH. Endothelial nitric oxide synthase limits left ventricular remodeling after myocardial infarction in mice. Circulation 2001;104:1286–1291.

106. Gaballa MA, Goldman S. Overexpression of endothelial nitric oxide synthase reverses the diminished vasorelaxation in the hindlimb vasculature in ischemic heart failure in vivo. J Mol Cell Cardiol 1999;31:1243–1252.

107. Guo X, Saini HK, Wang J, Gupta SK, Goyal RK, Dhalla NS. Prevention of remodeling in congestive heart failure due to myocardial infarction by blockade of the renin-angiotensin system. Expert Rev Cardiovasc Ther 2005;3:717-732.

108. Chatterjee K. Neurohormonal activation in congestive heart failure and the role of vasopressin. Am J Cardiol 2005;95:8B-13B.

109. Packer M, Lee WH, Kessler PD. Role of neurohormonal mechanisms in determining survival in patients with severe chronic heart failure. Circulation 1987;75 (suppl 4):80–92.

110. Thibonnier M. Vasopressin receptor antagonists in heart failure. Curr Opin Pharamacol 2003;3:683–687.

111. Ellison D, Schrier RW. The edematous patient: cardiac failure, cirrhosis, and nephrotic syndrome. In: Schrier RW, ed. Manual of Nephrology. Philadelphia: Lippincott Williams & Wilkins, 2000:1–36.

112. Lavoie JL, Sigmund CD. Minireview: overview of the renin-angiotensin system - an endocrine and paracrine system. Endocrinology 2003;144:2179–2183.

113. Schunkert H, Jackson B, Tang SS, Schoen FJ, Smits JF, Apstein CS, Lorell BH. Distribution and functional significance of cardiac angiotensin converting enzyme in hypertrophied rat hearts. Circulation 1993;87:1328–1339.

114. Weber KT, Brilla CG. Pathological hypertrophy and the cardiac interstitium: Fibrosis and the renin-angiotensin-aldosterone system. Circulation 1991;83:1849–1865.

115. Ollivier JP, Bouchet VA. Prospects for cardioreparation. Am J Cardiol 1992;70:27C–36C.

116. Bonvalet JP, Alfaidy N, Farman N, Lombes M. Aldosterone: Intracellular receptors in human heart. Eur Heart J 1995;16 (Suppl):92–97.

117. MizunoY, Yoshimura M, Yasue H, Sakamoto T, Ogawa H, Kugiyama K, Harada

E, Nakayama M, Nakamura S, Ito T, Shimasaki Y, Saito Y, Nakao K. Aldosterone production is activated in failing ventricle in humans. Circulation 2001;103:72–77.

118. Brilla CG, Matsubara LS, Weber KT. Anti-aldosterone treatment and the prevention of myocardial fibrosis in primary and secondary hyperaldosteronism. J Mol Cell Cardiol 1993;25:563-575.

119. Weber Kt. Efficacy of aldosterone receptor antagonism in heart failure: potential mechanism. Curr Heart Fail Rep 2004;1:51-56.

120. Creager MA, Faxon DP, Cutler SS, Kohlmann O, Ryan TJ, Gavras H. Contribution of vasopressin to vasoconstriction in patients with congestive heart failure: comparison with the renin-angiotensin system and the sympathetic nervous system. J Am Coll Cardiol 1986;7:758–765.

121. Thibonnier M, Coles P, Thibonnier A, Shoham M. The basic and clinical pharmacology of nonpeptide vasopressin receptor antagonists. Annu Rev Pharmacol Toxicol 2001;41:175–202.

122. Francis GS, Benedict C, Johnstone DE, Kirlin PC, Nicklas J, Liang CS, Kubo SH, Rudin-Toretsky E, Yusuf S. Comparison of neuroendocrine activation in patients with left ventricular dysfunction with and without congestive heart failure: a substudy of the Studies of Left Ventricular Dysfunction (SOLVD). Circulation 1990;82:1724 –1729.

123. Goldsmith SR, Francis GS, Cowley AW Jr, Levine TB, Cohn JN. Increased plasma arginine vasopressin levels in patients with congestive heart failure. J Am Coll Cardiol 1983:1:1385–1390.

124. Szatalowicz VL, Arnold PE, Chaimovitz C, Bichet D, Berl T, Schrier RW. Radioimmunoassay of plasma arginine vasopressin in hyponatremic patients with congestive heart failure. N Engl J Med 1981:305:263–266.

125. Struthers AD. Ten years of natriuretic peptide research: a new dawn for their diagnostic and therapeutic use? Brit Med J 1994;308:1615-1619.

126. Lang CC, Choy AM, Struthers AD. Atrial and brain natriuretic peptides: a dual natriuretic peptide system potentially involved in circulatory homeostasis. Clin Sci (Lond) 1992;83:519-527.

127. Levin ER. Endothelins. N Engl J Med 1995;333:356-363.

128. Watanabe T, Suzuki N, Shimamoto N, Fujino M, Imada A. Endothelin in myocardial infarction. Nature 1990;344:114.

129. Nguyen QT, Cernacek P, Calderoni A, Stewart DJ, Picard P, Sirois P, White M, Rouleau JL. Endothelin A receptor blockade causes adverse left ventricular remodeling but improves pulmonary artery pressure after infarction in the rat. Circulation 1998;98:2323–2330.

130. Kobayashi T, Miyauchi T, Sakai S, Kobayashi M, Yamaguchi I, Goto K, Sugishita Y. Expression of endothelin-1, ETA and ETB receptors, and ECE and distribution of endothelin-1 in failing rat heart. Am J Physiol Heart Circ Physiol 1999;276:H1197–H1206.

131. Piacentini L, Gray M, Honbo NY, Chentoufi J, Bergman M, Karliner JS. Endothelin-1 stimulates cardiac fibroblast proliferation through activation of protein kinase C. J Mol Cell Cardiol 2000;32:565–576.

132. Teerlink JR. Reversal of left ventricular remodeling: role of the endothelin

pathway. J Card Fail 2002;8 (6 Suppl):S494-S499.

133. Dhalla NS, Dixon IM, Rupp H, Barwinsky J. Experimental congestive heart failure due to myocardial infarction: sarcolemmal receptors and cation transporters. Basic Res Cardiol 1991;86 Suppl 3:13-23.

134. Holubarsch C, Hasenfuss G, Thierfelder L, Pieske B, Just H. The heart in heart failure. Ventricular and myocardial alterations. Eur Heart J 1991;12 Suppl C:8-13.

135. Langer GA. Calcium at the sarcolemma. J Mol Cell Cardiol 1984;16:147-153.

136. Dhalla NS, Ziegelhoffer A, Harrow JA. Regulatory role of membrane systems in heart function. Can J Physiol Pharmacol 1977;55:1211-1234.

137. Dhalla NS, Das PK, Sharma GP. Subcellular basis of cardiac contractile failure. J Mol Cell Cardiol 1978;10:363-385.

138. Dhalla NS, Pierce GN, Panagia V, Singal PK, Beamish RE. Calcium movements in relation to heart function. Basic Res Cardiol 1982;77:117-139.

139. Dixon IM, Lee SL, Dhalla NS. Nitrendipine binding in congestive heart failure due to myocardial infarction. Circ Res 1990;66:782-788.

140. Takahashi T, Allen PD, Lacro RV, Marks AR, Dennis AR, Schoen FJ, Grossman W, Marsh JD, Izumo S. Expression of dihydropyridine receptor (Ca^{2+} channel) and calsequestrin genes in the myocardium of patients with end-stage heart failure. J Clin Invest 1992;90:927-935.

141. Wagner JA, Weisman HF, Snowman AM, Reynolds IJ, Weisfeldt ML, Snyder SH. Alterations in calcium antagonist receptors and sodium-calcium exchange in cardiomyopathic hamster tissues. Circ Res 1989;65:205-214.

142. Dixon IM, Hata T, Dhalla NS. Sarcolemmal Na^+-K^+-ATPase activity in congestive heart failure due to myocardial infarction. Am J Physiol Cell Physiol 1992;262:C664-C671.

143. Dixon IM, Hata T, Dhalla NS. Sarcolemmal calcium transport in congestive heart failure due to myocardial infarction in rats. Am J Physiol Heart Circ Physiol 1992;262:H1387-H1394.

144. Makino N, Jasmin G, Beamish RE, Dhalla NS. Sarcolemmal Na^+-Ca^{2+} exchange during the development of genetically determined cardiomyopathy. Biochem Biophys Res Commun 1985;133:491-497.

145. Balasubramanian V, McNamara DB, Singh JN, Dhalla NS. Biochemical basis of heart function. X. Reduction in the Na^+-K^+-stimulated ATPase activity in failing rat heart due to hypoxia. Can J Physiol Pharmacol 1973;51:504-510.

146. Panagia V, Singh JN, Anand-Srivastava MB, Pierce GN, Jasmin G, Dhalla NS. Sarcolemmal alterations during the development of genetically determined cardiomyopathy. Cardiovasc Na^+-K^+-ATPase activity in the failing rabbit heart. Jpn Heart J 1972;13:73-83.

147. Yazaki Y, Fujii J. Depressed Na^+-K^+-ATPase activity in the fialing rabbit heart. Jpn Heart J 1972;13:73-83

148. Khatter JC, Prasad K. Myocardial sarcolemmal ATPase in dogs with induced mitral insufficiency. Cardiovasc Res 1976;10:637-641.

149. Prasad K, Khatter JC, Bharadwaj B. Intra- and extracellular electrolytes and sarcolemmal ATPase in the failing heart due to pressure overload in dogs. Cardiovasc Res 1979;13:95-104.

150. Johns TN, Olson BJ. Experimental myocardial infarction. I. A method of coronary occlusion in small animals. Ann Surg 1954;140:675-682.

151. Inui M, Saito A, Fleischer S. Isolation of the ryanodine receptor from cardiac sarcoplasmic reticulum and identity with the feet structures. J Biol Chem 1987;262:15637-15642.

152. Afzal N, Dhalla NS. Differential changes in left and right ventricular SR calcium transport in congestive heart failure. Am J Physiol Heart Circ Physiol 1992;262:H868-H874.

153. Zarain-Herzberg A, Afzal N, Elimban V, Dhalla NS. Decreased expression of cardiac sarcoplasmic reticulum Ca^{2+}-pump ATPase in congestive heart failure due to myocardial infarction. Mol Cell Biochem 1996;163-164:285-290.

154. Rupp H, Elimban V, Dhalla NS. Diabetes-like action of intermittent fasting on sarcoplasmic reticulum Ca^{2+}-pump ATPase and myosin isoenzymes can be prevented by sucrose. Biochem Biophys Res Commun 1989;164:319-325.

155. Afzal N, Pierce GN, Elimban V, Beamish RE, Dhalla NS. Influence of verapamil on some subcellular defects in diabetic cardiomyopathy. Am J Physiol Endocrinol Met 1989;256:E453-E458.

156. Musch TI, Moore RL, Smaldone PG, Riedy M, Zelis R. Cardiac adaptations to endurance training in rats with a chronic myocardial infarction. J Appl Physiol 1989;66:712-719.

157. Mercadier JJ, Lompre AM, Wisnewsky C, Samuel JL, Bercovici J, Swynghedauw B, Schwartz K. Myosin isoenzyme changes in several models of rat cardiac hypertrophy. Circ Res 1981;49:525-532.

158. Chevalier B, Callens F, Charlemagne D, Delcayre C, Lompre AM, Lelievre L, Mercadier JJ, Moalic JM, Mansier P, Rappaport L, Samuel JL, Schwartz K, Swynghedauw B. Signal and adaptational changes in gene expression during cardiac overload. J Mol Cell Cardiol 1989;21 Suppl 5:71-77.

159. Dhalla NS, Elimban V, Rupp H, Takeda N, Nagano M. Role of calcium in cardiac cell damage and dysfunction. In: Sperelakis N, editor: Physiology and Pathophysiology of the Heart. 3rd ed. Boston: Kluwer Academic Publishers, 1995;605-623.

160. Scheuer J. Metabolism of the heart in cardiac failure. Prog Cardiovasc Dis 1970;13:24-54.

161. Ingwall JS, Weiss RG. Is the failing heart energy starved? On using chemical energy to support cardiac function. Circ Res 2004;95:135-145.

162. Neubauer S, Horn M, Naumann A, Tian R, Hu K, Laser M, Friedrich J, Gaudron P, Schnackerz K, Ingwall JS, Ertl G. Impairment of energy metabolism in intact residual myocardium of rat hearts with chronic myocardial infarction. J Clin Invest 1995;95:1092-1100.

163. Dixon IMC, Dhalla NS. Alterations in cardiac adrenoceptors in congestive heart failure secondary to myocardial infarction. Cor Art Dis 1991;2:805-814.

164. Bohm M, Beuckelmann D, Brown L, Feiler G, Lorenz B, Nabauer M, Kemkes B, Erdmann E. Reduction of beta-adrenoceptor density and evaluation of positive inotropic responses in isolated, diseased human myocardium. Eur Heart J 1988;9:844-852.

165. Yamamoto J, Ohyanagi M, Morita M, Iwasaki T. Beta-adrenoceptor-G protein-

adenylate cyclase complex in rat hearts with ischemic heart failure produced by coronary artery ligation. J Mol Cell Cardiol 1994;26:617-626.

166. Sethi R, Bector N, Takeda N, Nagano M, Jasmin G, Dhalla NS. Alterations in G-proteins in congestive heart failure in cardiomyopathic (UM-X7.1) hamsters. Mol Cell Biochem 1994;140:163-170.

167. McDonald KM, D'Aloia A, Parrish T, Mock J, Hauer K, Stillman AE, Cohn JN. Functional impact of an increase in ventricular mass after myocardial damage and its attenuation by converting enzyme inhibition. J Card Fail 1998;4:203–212.

168. Greenberg B, Quinones MA, Koilpillai C, Limacher M, Shindler D, Benedict C, Shelton B. Effects of long-term enalapril therapy on cardiac structure and function in patients with left ventricular dysfunction. Results of the SOLVD echocardiography substudy. Circulation 1995;91:2573–2581.

169. Ohnishi A, Orita Y, Takagi N, Fujita T, Toyoki T, Ihara Y, Yamamura Y, Inoue T, Tanaka T. Aquaretic effect of a potent, orally active, nonpeptide V2 antagonist in men. J Pharmacol Exp Ther 1995;272:546–551.

170. Clair MJ, King MK, Goldberg AT. Selective vasopressin, angiotensin II, or dual receptor blockade with developing congestive heart failure. J Pharmacol Exp Ther 2000:293:852–860.

171. Packer M. Pathophysiology of chronic heart failure. Lancet 1992;340:88-92.

172. Sabbah HN. The cellular and physiologic effects of beta-blockers in heart failure. Clin Cardiol (Suppl 5)1999;22:V16-V20.

173. Beta-Blocker Heart Attack Trial (BHAT) Research Group. A randomized trial of propranolol in patients with acute myocardial infarction. I. Mortality results.

JAMA 1982; 247:1707-1714.

174. Yusuf S, Peto R, Lewis J, Collins R, Sleight P. Beta blockade during and after myocardial infarction: an overview of the randomized trials. Prog Cardiovasc Dis 1985;27:335-371.

175. Andersson B, Blomstrom-Lundqvist C, Hedner T, Waagstein F. Exercise hemodynamics and myocardial metabolism during long-term beta-adrenergic blockade in severe heart failure. J Am Coll Cardiol 1991;18:1059-1066.

176. Weksler BB, Gillick M, Pink J. Effect of propranolol on platelet function. Blood 1977;49:185-196.

177. Swedberg K, Hjalmarson A, Waagstein F, Wallentin I. Adverse effects of beta-blockade withdrawal in patients with congestive cardiomyopathy. Br Heart J 1980;44:134-142.

178. Frishman WH, Lazar EJ. Reduction of mortality, sudden death and non-fatal reinfarction with beta-adrenergic blockers in survivors of acute myocardial infarction: a new hypothesis regarding the cardioprotective action of beta-adrenergic blockade. Am J Cardiol 1990;66:66G-70G.

179. Bristow MR. Beta-adrenergic receptor blockade in chronic heart failure. Circulation 2000; 101:558-569.

180. Sabbah HN, Sharov VG, Gupta RC, Todor A, Singh V, Goldstein S. Chronic therapy with metoprolol attenuates cardiomyocyte apoptosis in dogs with heart failure. J Am Coll Cardiol 2000;36:1698-1705.

181. Heilbrunn SM, Shah P, Bristow MR, Valantine HA, Ginsburg R, Fowler MB. Increased beta-receptor density and improved hemodynamic response to

catecholamine stimulation during long-term metoprolol therapy in heart failure from dilated cardiomyopathy. Circulation 1989;79:483-490.

182. Matsui S, Fu ML. Prevention of experimental autoimmune cardiomyopathy in rabbits by receptor blockers. Autoimmunity 2001;34:217-220.

183. Newton GE, Parker JD. Acute effects of beta 1-selective and nonselective beta-adrenergic receptor blockade on cardiac sympathetic activity in congestive heart failure. Circulation 1996;94:353-358.

184. Sanderson JE, Chan SK, Yip G, Yeung LY, Chan KW, Raymond K, Woo KS. Beta-blockade in heart failure. A comparison of carvedilol with metoprolol. J Am Coll Cardiol 1999;34:1522–1528.

185. Metra M, Giubbini R, Nodari S, Boldi E, Modena MG, Dei Cas L. Differential effects of β-blockers in patients with heart failure. A prospective, randomized, double-blind comparison of the long-term effects of metoprolol versus carvedilol. Circulation 2000;102:546–551.

186. Australia/New Zealand Heart Failure Research Collaborative Group. Randomised, placebo-controlled trial of carvedilol in patients with congestive heart failure due to ischaemic heart disease. Lancet 1997;349:375–380.

187. Pitt B. "Escape" of aldosterone production in patients with left ventricular dysfunction treated with an angiotensin converting enzyme inhibitor: implications for therapy. Cardiovasc Drugs Ther 1995;9:145–149.

188. Tsutamoto T, Wada A, Maeda K, Mabuchi N, Hayashi M, Tsutsui T, Ohnishi M, Sawaki M, Fujii M, Matsumoto T, Matsui T, Kinoshita M. Effect of spironolactone on plasma brain natriuretic peptide and left ventricular

remodeling in patients with congestive heart failure. J Am Coll Cardiol 2001;37:1228–1233.

189. Dawson A, Davies JI, Struthers AD. The role of aldosterone in heart failure and the clinical benefits of aldosterone blockade. Expert Rev Cardiovasc Ther 2004;2:29-36.

190. Mann DL, Deswal A, Bozkurt B, Torre-Amione G. New therapeutics for chronic heart failure. Annu Rev Med 2002;53:59-74.

191. Burnett JC Jr. 1999. Vasopeptidase inhibition: a new concept in blood pressure management. J. Hypertens Suppl 17:S37–S43.

192. McClean DR, Ikram H, Garlick AH, Richards AM, Nicholls MG, Crozier IG. The clinical, cardiac, renal, arterial and neurohormonal effects of omapatrilat, a vasopeptidase inhibitor, in patients with chronic heart failure. J Am Coll Cardiol 2000;36:479–486.

193. Trippodo NC, Fox M, Monticello TM, Panchal BC, Asaad MM. Vasopeptidase inhibition with omapatrilat improves cardiac geometry and survival in cardiomyopathic hamsters more than does ACE inhibition with captopril. J Cardiovasc Pharmacol 1999;34:782-790.

194. Piano MR, Kim SD, Jarvis C. Cellular events linked to cardiac remodeling in heart failure: targets for pharmacologic intervention. J Cardiovasc Nurs 2000;14:1-23.

195. Deswal A, Bozkurt B, Seta Y, Parilti-Eiswirth S, Hayes FA, Blosch C, Mann DL. Safety and efficacy of a soluble P75 tumor necrosis factor receptor (Enbrel, etanercept) in patients with advanced heart failure. Circulation 1999 29;99:3224–

3226.

196. Bozkurt B, Torre-Amione G, Smith, Warren MS, Whitmore J, Soran OZ, Feldman AM, Mann DL. Results of targeted anti-tumor necrosis factor therapy with etanercept (ENBREL) in patients with advanced heart failure. Circulation 2001;103:1044–1047.

197. Bauersachs J, Galuppo P, Fraccarollo D, Christ M, Ertl G. Improvement of left ventricular remodeling and function by hydroxymethylglutaryl coenzyme a reductase inhibition with cerivastatin in rats with heart failure after myocardial infarction. Circulation. 2001;104:982-985.

198. Hayashidani S, Tsutsui H, Shiomi T, Suematsu N, Kinugawa S, Ide T, Wen J, Takeshita A. Fluvastatin, a 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibitor, attenuates left ventricular remodeling and failure after experimental myocardial infarction. Circulation. 2002;105:868-873.

199. Reddy R, Chahoud G, Mehta JL. Modulation of cardiovascular remodeling with statins: fact or fiction? Curr Vasc Pharmacol 2005;3:69-79.

200. Ichiki T, Takeda K, Tokunou T, Iino N, Egashira K, Shimokawa H, Hirano K, Kanaide H, Takeshita A. Downregulation of angiotensin II type 1 receptor by hydrophobic 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors in vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 2001;21:1896-1901.

201. Kumar D, Jugdutt BI. Apoptosis and oxidants in the heart. J Lab Clin Med 2003;142:288-297.

202. Laufs U, La Fata V, Plutzky J, Liao JK. Upregulation of endothelial nitric oxide

synthase by HMG CoA reductase inhibitors. Circulation 1998;97:1129-1135.

203. Kalinowski L, Dobrucki LW, Brovkovych V, Malinski T. Increased nitric oxide bioavailability in endothelial cells contributes to the pleiotropic effect of cerivastatin. Circulation 2002;105:933-938.

204. Pliquett RU, Cornish KG, Peuler JD, Zucker IH. Simvastatin normalizes autonomic neural control in experimental heart failure. Circulation 2003;107:2493–2498.

205. Hernandez-Perera O, Perez-Sala D, Soria E, Lamas S. Involvement of Rho GTPases in the transcriptional inhibition of preproendothelin-1 gene expression by simvastatin in vascular endothelial cells. Circ Res 2000;87:616-622.

206. Krum H, McMurray JJ. Statins and chronic heart failure: do we need a large-scale outcome trial? J Am Coll Cardiol 2002;39:1567-1573.

207. Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: a randomised placebo-controlled trial. Lancet 2002;360:7-22.

208. Mishima T, Tanimura M, Suzuki G, Todor A, Sharov VG, Goldstein S, Sabbah HN. Effects of long-term therapy with bosentan on the progression of left ventricular dysfunction and remodeling in dogs with heart failure. J Am Coll Cardiol 2000;35:222–229.

209. Spinale FG,Walker JD, Mukherjee R, Iannini JP, Keever AT, Gallagher KP. Concomitant endothelin receptor subtype-A blockade during the progression of pacing-induced congestive heart failure in rabbits. Circulation 1997;95:1918–1929.

210. Mulder P, Richard V, Bouchart F, Derumeaux G, Munter K, Thuillez C. Selective ETA receptor blockade prevents left ventricular remodeling and detioration of cardiac function in experimental heart failure. Cardiovasc Res 1998;39:600–608.

211. Mulder P, Boujedaini H, Richard V, Derumeaux G, Henry JP, Renet S, Wessale J, Opgenorth T, Thuillez C. Selective endothelin-A versus combined endothelin-A/endothelin-B receptor blockade in rat chronic heart failure. Circulation 2000;102:491–493.

212. Hu K, Gaudron P, Schmidt TJ, Hoffmann KD, Ertl G. Aggravation of left ventricular remodeling by a novel specific endothelin ETA antagonist EMD94246 in rats with experimental myocardial infarction. J Cardiovasc Pharmacol 1998;32:505–508.

213. Fraccarollo D, Hu K, Galuppo P, Gaudron P, Ertl G. Chronic endothelin receptor blockade attenuates progressive ventricular dilation and improves cardiac function in rats with myocardial infarction. Circulation 1997;96:3963–3973.

214. Sakai S, Miyauchi T, Kobayashi M, Yamaguchi I, Goto K, Sugishita Y. Inhibition of myocardial endothelin pathway improves long-term survival in heart failure. Nature 1996;384:353–355.

215. Mulder P, Richard V, Derumeaux G, Hogie M, Henry JP, Lallemand F, Compagnon P, Mace B, Comoy E, Letac B, Thuillez C. Role of endogenous endothelin in chronic heart failure: effect of long-term treatment with an endothelin antagonist on survival, hemodynamics, and cardiac remodeling. Circulation 1997;96:1976–1982.

216. Fraccarollo D, Hu K, Galuppo P, Gaudron P, Ertl G. Chronic endothelin receptor blockade attenuates progressive ventricular dilation and improves cardiac function in rats with myocardial infarction: possible involvement of myocardial endothelin system in ventricular remodeling. Circulation 1997;96:3963–3973.

217. Oie E, Bjonerheim R, Grogaard HK, Kongshaug H, Smiseth OA, Attramadal H. ET-receptor antagonism, myocardial gene expression, and ventricular remodeling during CHF in rats. Am J Physiol Heart Circ Physiol 1998;275:H868–H877.

218. Schafhalter-Zoppoth I, Teerlink JR. Endothelin antagonism with bosentan improves myocardial mechanics and ventricular remodeling in rats with chronic heart failure. J Am Coll Cardiol 2002;39:171A.

219. Schafhalter-Zoppoth I, Gray MO, Teerlink JR. Endothelin antagonism with bosentan, a dual receptor blocker, normalizes dysregulated transforming growth factor beta signaling pathways in rats with chronic heart failure. J Am Coll Cardiol 2002;39:168A.

220. Iwanaga Y, Kihara Y, Inagaki K, Onozawa Y, Yoneda T, Kataoka Y, Sasayama S. Differential effects of angiotensin II versus endothelin-1 inhibitions in hypertrophic left ventricular myocardium during transition to heart failure. Circulation 2001;104:606–612.

221. Mishima T, Tanimura M, Suzuki G, Todor A, Sharov VG, Goldstein S, Sabbah HN. Effects of long-term therapy with bosentan on the progression of left ventricular dysfunction and remodeling in dogs with heart failure. J Am Coll Cardiol 2000;35:222–229.

222. Tavazzi L. Epidemiology of dilated cardiomyopathy: a still undetermined entity.

Eur Heart J 1997;18:4-6.

223. Keck BM, Bennett LE, Rosendale J, Daily OP, Novick RJ, Hosenpud JD. Worldwide thoracic organ transplantation: a report from the UNOS/ISHLT International Registry for Thoracic Organ Transplantation. Clin Transpl 1999;35-49.

224. Batista RJ, Santos JL, Takeshita N, Bocchino L, Lima PN, Cunha MA. Partial left ventriculectomy to improve left ventricular function in end-stage heart disease. J Card Surg 1996;11:96-97.

225. Batista RJ, Verde J, Nery P, Bocchino L, Takeshita N, Bhayana JN, Bergsland J, Graham S, Houck JP, Salerno TA. Partial left ventriculectomy to treat end-stage heart disease. Ann Thorac Surg 1997;64:634-638.

226. Schreuder JJ, Steendijk P, van der Veen FH, Alfieri O, van der Nagel T, Lorusso R, van Dantzig JM, Prenger KB, Baan J, Wellens HJ, Batista RJ. Acute and short-term effects of partial left ventriculectomy in dilated cardiomyopathy: assessment by pressure-volume loops. J Am Coll Cardiol 2000;36:2104-2114.

227. Dickstein ML, Spotnitz HM, Rose EA, Burkhoff D. Heart reduction surgery: an analysis of the impact on cardiac function. J Thorac Cardiovasc Surg 1997;113:1032-1040.

228. Franco-Cereceda A, McCarthy PM, Blackstone EH, Hoercher KJ, White JA, Young JB, Starling RC. Partial left ventriculectomy for dilated cardiomyopathy: is this an alternative to transplantation? J Thorac Cardiovasc Surg 2001;121:879-893.

229. Dor V. The endoventricular circular patch plasty ("Dor procedure") in ischemic

akinetic dilated ventricles. Heart Fail Rev 2001;6:187-193.

230. Dor V, Sabatier M, Di Donato M, Montiglio F, Toso A, Maioli M. Efficacy of endoventricular patch plasty in large postinfarction akinetic scar and severe left ventricular dysfunction: comparison with a series of large dyskinetic scars. J Thorac Cardiovasc Surg 1998;116:50-59.

231. Pagano D, Bonser RS, Camici PG. Myocardial revascularization for the treatment of post-ischemic heart failure. Curr Opin Cardiol 1999;14:506-509.

232. La Canna G, Alfieri O, Giubbini R, Gargano M, Ferrari R, Visioli O. Echocardiography during infusion of dobutamine for identification of reversibly dysfunction in patients with chronic coronary artery disease. J Am Coll Cardiol 1994;23:617-626.

233. Alfieri O, La Canna G, Giubbini R, Pardini A, Zogno M, Fucci C. Recovery of myocardial function. The ultimate target of coronary revascularization. Eur J Cardiothorac Surg 1993;7:325-330.

234. Bolling SF, Deeb GM, Brunsting LA, Bach DS. Early outcome of mitral valve reconstruction in patients with end-stage cardiomyopathy. J Thorac Cardiovasc Surg 1995;109:676-682.

235. Grigioni F, Enriquez-Sarano M, Zehr KJ, Bailey KR, Tajik AJ. Ischemic mitral regurgitation: long-term outcome and prognostic implications with quantitative Doppler assessment. Circulation 2001;103:1759-1764.

236. Zeltsman D, Acker MA. Surgical management of heart failure: an overview. Annu Rev Med 2002;53:383-391.

237. Hendren WG, Nemec JJ, Lytle BW, Loop FD, Taylor PC, Stewart RW,

Cosgrove DM 3rd. Mitral valve repair for ischemic mitral insufficiency. Ann Thorac Surg 1991;52:1246-1251.

238. Bolling SF, Pagani FD, Deeb GM, Bach DS. Intermediate-term outcome of mitral reconstruction in cardiomyopathy. J Thorac Cardiovasc Surg 1998 Feb;115:381-386.

239. Smolens IA, Pagani FD, Bolling SF. Mitral valve repair in heart failure. Eur J Heart Fail 2000;2:365-371.

240. Badhwar V, Bolling SF. Mitral valve surgery in the patient with left ventricular dysfunction. Semin Thorac Cardiovasc Surg 2002;14:133-136.

241. Alfieri O, Maisano F, De Bonis M, Stefano PL, Torracca L, Oppizzi M, La Canna G. The double-orifice technique in mitral valve repair: a simple solution for complex problems. J Thorac Cardiovasc Surg 2001;122:674-681.

242. Sintek CF, Pfeffer TA, Kochamba G, Fletcher A, Khonsari S. Preservation of normal left ventricular geometry during mitral valve replacement. J Heart Valve Dis 1995;4:471-475.

243. Sarris GE, Cahill PD, Hansen DE, Derby GC, Miller DC. Restoration of left ventricular systolic performance after reattachment of the mitral chordae tendineae. The importance of valvular-ventricular interaction. J Thorac Cardiovasc Surg 1988;95:969-979.

244. Natsuaki M, Itoh T, Tomita S, Furukawa K, Yoshikai M, Suda H, Ohteki H. Importance of preserving the mitral subvalvular apparatus in mitral valve replacement. Ann Thorac Surg 1996;61:585-590.

245. Komeda M, David TE, Rao V, Sun Z, Weisel RD, Burns RJ. Late hemodynamic

effects of the preserved papillary muscles during mitral valve replacement. Circulation 1994;90:II190-II194.

246. Pappone C, Rosanio S, Oreto G, Tocchi G, Gugliotta F, Vicedomini G, Salvati A, Dicandia C, Mazzone P, Santinelli V, Gulletta S, Chierchia S. Circumferential radiofrequency ablation of pulmonary vein ostia: a new anatomic approach for curing atrial fibrillation. Circulation 2000;102:2562–2564.

247. Alfieri O, Benussi S. Mitral valve surgery with concomitant atrial fibrillation. Cardiol Rev 2000;8:317–321.

248. Chachques JC, Grandjean PA, Carpentier A. Latissimus dorsi dynamic cardiomyoplasty. Ann Thorac Surg 1989;47:600–604.

249. James KB, McCarthy PM, Thomas JD, Vargo R, Hobbs RE, Sapp S, Bravo E. Effect of implantable left ventricular device on neuroendocrine activation in heart failure. Circulation 1995;92(suppl):II-191–II-195.

250. Konertz W, Dushe S, Hotz H, Braun JP, Spiess C, Endzweiler C, Stantke K, Sapsford E, Sabbah H, Kleber FX. Safety and feasibility of a cardiac support device. J Card Surg 2001;16:113-117.

251. Margulies KB. Reversal mechanisms of left ventricular remodeling: lessons from left ventricular assist device experiments. J Card Fail 2002;8(6 Suppl):S500-S505.

252. Nakatani S, McCarthy P, Kottke-Marchaant K, Harasaki H, James KB, Savage R, Thomas JD. Left ventricular echocardiographic and histologic changes: impact of chronic unloading by an implantable ventricular assist device. J Am Coll Cardiol 1996;27:894–901.

253. Altemose GT, Gritsus V, Jeevanandam V, Goldman B, Margulies KB. Altered myocardial phenotype after mechanical support in human beings with advanced cardiomyopathy. J Heart Lung Transplant 1997;16:765–773.

254. McCarthy PM, James KB, Savage RM, Vargo R, Kendall K, Harasaki H, Hobbs RE, Pashkow FJ. Implantable left ventricular assist device. Approaching an alternative for end-stage heart failure. Implantable LVAD Study Group. Circulation 1994;90:II83-86.

255. Zafeiridis A, Jeevanandam V, Houser SR, Margulies KB. Regression of cellular hypertrophy after left ventricular assist device support. Circulation 1998;98:656–662.

256. Kinoshita M, Takano H, Takaichi S, Taenaka Y, Nakatani T. Influence of prolonged ventricular assistance on myocardial histopathology in intact heart. Ann Thorac Surg 1996;61:640–645.

257. Barbone A, Holmes J, Heerdt P, The'A, Naka Y, Joshi N, Daines M, Marks A, Oz M, Burkhoff D. Comparison of right and left ventricular response to left ventricular assist device support in patients with severe heart failure: a primary role of mechanical unloading underlying reverse remodeling. Circulation 2001;104:670–675.

258. Bruckner B, Stetson S, Perez-Verdia A, Youker K, Radovancevic B, Connelly J, Koerner M, Entman M, Frazier O, Noon G, Torre-Amione G. Regression of fibrosis and hypertrophy in failing myocardium following mechanical circulatory support. J Heart Lung Transplant 2001;20:457–464.

259. Frazier OH, Benedict CR, Radovancevic B, Bick RJ, Capek P, Springer WE,

Marcis MP, Delgado R, Buja LM. Improved left ventricular function after chronic left ventricular unloading. Ann Thorac Surg 1996;62:675–682.

260. Heerdt PM, Holmes JW, Cai B, Barbone A, Madigan JD, Reiken S, Lee DL, Oz MC, Marks AR, Burkhoff D. Chronic unloading by left ventricular assist device reverses contractile dysfunction and alters gene expression in end-stage heart failure. Circulation 2000;102:2713–2719.

261. Tomaselli GF, Beuckelmann DJ, Calkins HG, Berger RD, Kessler PD, Lawrence JH, Kass D, Feldman AM, Marban E. Sudden death in cardiac failure: the role of abnormal repolarization. Circulation 1994;90:2534–2539.

262. Bakker PF, Meijburg HW, de Vries JW, Mower MM, Thomas AC, Hull ML, Robles De Medina EO, Bredee JJ. Biventricular pacing in end-stage heart failure improves functional capacity and left ventricular function. J Interv Card Electrophysiol 2000;4:395–404.

263. Abraham WT, Fisher WG, Smith AL, Delurgio DB, Leon AR, Loh E, Kocovic DZ, Packer M, Clavell AL, Hayes DL, Ellestad M, Trupp RJ, Underwood J, Pickering F, Truex C, McAtee P, Messenger J. Cardiac resynchronization in chronic heart failure. N Engl J Med 2002;346:1845–1853.

264. Linde C, Leclercq C, Rex S, Garrigue S, Lavergne T, Cazeau S, McKenna W, Fitzgerald M, Deharo JC, Alonso C, Walker S, Braunschweig F, Bailleul C, Daubert JC. Long-term benefits of biventricular pacing in congestive heart failure: results from the MUltisite STimulationin cardiomyopathy (MUSTIC) study. J Am Coll Cardiol 2002;40:111–118.

265. Cazeau S, Leclercq C, Lavergne T, Walker S, Varma C, Linde C, Garrigue S,

Kappenberger L, Haywood GA, Santini M, Bailleul C, Daubert JC. Effects of multisite biventricular pacing in patients with heart failure and intraventricular conduction delay. N Engl J Med 2001; 344:873–880.

266. Auricchio A, Stellbrink C, Sack S, Block M, Vogt J, Bakker P, Huth C, Schondube F, Wolfhard U, Bocker D, Krahnefeld O, Kirkels H. Long-term clinical effect of hemodynamically optimized cardiac resynchronization therapy in patients with heart failure and ventricular conduction delay. J Am Coll Cardiol 2002;39:2026–2033.

267. Braunschweig F, Linde C, Gadler F, Ryden L. Reduction of hospital days by biventricular pacing. Eur J Heart Fail 2000;2:399–406.

268. Grines CL, Bashore TM, Boudoulas H, Olson S, Shafer P, Wooley CF. Functional abnormalities in isolated left bundle branch block. The effect of interventricular asynchrony. Circulation 1989;79:845–853.

269. Xiao HB, Brecker SJ, Gibson DG. Differing effects of right ventricular pacing and left bundle branch block on left ventricular function. Br Heart J 1993;69:166–173.

270. Aaronson KD, Schwartz JS, Chen TM, Wong KL, Goin JE, Mancini DM. Development and prospective validation of a clinical index to predict survival in ambulatory patients referred for cardiac transplant evaluation. Circulation 1997; 95:2660–2667.

271. Xiao HB, Roy C, Gibson DG. Nature of ventricular activation in patients with dilated cardiomyopathy: evidence for bilateral bundle branch block. Br Heart J 1994;72:167–174.

272. Saxon LA, Kerwin WF, Cahalan MK, Kalman JM, Olgin JE, Foster E, Schiller NB, Shinbane JS, Lesh MD, Merrick SH. Acute effects of intraoperative multisite ventricular pacing on left ventricular function and activation/contraction sequence in patients with depressed ventricular function. J Cardiovasc Electrophysiol 1998;9:13–21.

273. Blanc JJ, Etienne Y, Gilard M, Mansourati J, Munier S, Boschat J, Benditt DG, Lurie KG. Evaluation of different ventricular pacing sites in patients with severe heart failure: results of an acute hemodynamic study. Circulation 1997; 96:3273–3277.

274. Cohn JN. The management of chronic heart failure. N Engl J Med. 1996;335:490-498.

275. Pfeffer MA, Pfeffer JM, Fishbein MC, Fletcher PJ, Spadaro J, Kloner RA, Braunwald E. Myocardial infarct size and ventricular function in rats. Circ Res. 1979;44:503-512.

276. Teerlink JR, Goldhaber SZ, Pfeffer MA. An overview of contemporary etiologies of congestive heart failure. Am Heart J. 1991;121:1852-1853

277. Kostuk WJ, Kazamias TM, Gander MP, Simon AL, Ross J Jr. Left ventricular size after acute myocardial infarction: serial changes and their prognostic significance. Circulation 1973; 47:1174–1179.

278. Pfeffer MA, Braunwald E, Moye LA, et al. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction: results of the survival and ventricular enlargement trial. The SAVE Investigators. N Engl J Med 1992;327:669–677

279. Teerlink J, Massie B. Beta-adrenergic blocker mortality trials in congestive heart failure. Am J Cardiol 1999;84:94R–102R.

280. Cleland JG. Anticoagulant and antiplatelet therapy in heart failure. Curr Opin Cardiol. 1997;12:276-287

281. Loh E, Sutton MS, Wun CC, Rouleau JL, Flaker GC, Gottlieb SS, Lamas GA, Moye LA, Goldhaber SZ, Pfeffer MA. Ventricular dysfunction and the risk of stroke after myocardial infarction. N Engl J Med. 1997;336:251-257

282. Lip GY, Gibbs CR. Does heart failure confer a hypercoagulable state? Virchow's triad revisited. J Am Coll Cardiol. 1999;33:1424-1426.

283. Cleland JG, Massie BM, Packer M. Sudden death in heart failure: vascular or electrical? Eur J Heart Fail. 1999;1:41-45

284. Dangas G, Fuster V. Management of restenosis after coronary intervention. Am Heart J. 1996;132:428-436

285. Nguyen K, AurnesI, Kjekshus J. Interaction between enalapril and aspirin on mortality after acute myocardial infarction: subgroup analysis of the Cooperative New Scandinavian Enalapril Survival Study II (CONSENSUS II). Am J Cardiol 1997;79:115 –119

286. Al-Khadra A, Salem D, Rand W, Udelson J, Smith J, Konstam M. Warfarin anticoagulation and survival: a cohort analysis from the studies of left ventricular dysfunction. J Am Coll Cardiol 1998;31:749 –753

287. Haynes WG, Webb DJ. Endothelium-dependent modulation of responses to endothelin-I in human veins. Clin Sci (Lond). 1993; 84:427-433

288. Davie AP, Love MP, McMurray JJ. Even low-dose aspirin inhibits arachidonic

acid-induced vasodilation in heart failure. Clin Pharmacol Ther. 2000;67:530-537

289. Nakamura S, Hall P, Gaglione A, Tiecco F, Di Maggio M, Maiello L, Martini G, Colombo A. High pressure assisted coronary stent implantation accomplished without intravascular ultrasound guidance and subsequent anticoagulation. J Am Coll Cardiol 1997;29:21–27

290. Schomig A, Neumann FJ, Kastrati A, Schuhlen H, Blasini R, Hadamitzky M, Walter H, Zitzmann-Roth EM, Richardt G, Alt E, Schmitt C, Ulm K. A randomized comparison of antiplatelet and anticoagulant therapy after the placement of coronary-artery stents. N Engl J Med 1996;334:1084–1089

291. Gent M, Blakely JA, Easton JD, Ellis DJ, Hachinski VC, Harbison JW, Panak E, Roberts RS, Sicurella J, Turpie AG. The Canadian American ticlopidine study (CATS) in thromboembolic stroke. Lancet 1989;1:1215–1220

292. Hass WK, Easton JD, Adams HP Jr, Pryse-Phillips W, Molony BA, Anderson S, Kamm B. A randomized trial comparing ticlopidine hydrochloride with aspirin for the prevention of stroke in high-risk patients. N Engl J Med 1989;321:501–507

293. Scheuer J, Bhan AK. Cardiac contractile proteins. Adenosine triphosphatase activity and physiological function. Circ Res 1979;45:1-12.

294. Eisenberg E, Greene LE. The relation of muscle biochemistry to muscle physiology. Annu Rev Physiol 1980;42:293-309.

295. Schwartz K, Lecarpentier Y, Martin JL, Lompre AM, Mercadier JJ, Swynghedauw B. Myosin isoenzymic distribution correlates with speed of

myocardial contraction. J Mol Cell Cardiol 1981;13:1071-1075.

296. Mahdavi V, Periasamy M, Nadal-Ginard B. Molecular characterization of two myosin heavy chain genes expressed in the adult heart. Nature 1982;297:659-664.

297. Mahdavi V, Chambers AP, Nadal-Ginard B. Cardiac alpha- and beta-myosin heavy chain genes are organized in tandem. Proc Natl Acad Sci USA 1984;81:2626-2630.

298. Hoh JF, McGrath PA, Hale PT. Electrophoretic analysis of multiple forms of rat cardiac myosin: effects of hypophysectomy and thyroxine replacement. J Mol Cell Cardiol 1978;10:1053-1076.

299. Pope B, Hoh JF, Weeds A. The ATPase activities of rat cardiac myosin isoenzymes. FEBS Lett 1980;118:205-208.

300. Effron MB, Bhatnagar GM, Spurgeon HA, Ruano-Arroyo G, Lakatta EG. Changes in myosin isoenzymes, ATPase activity, and contraction duration in rat cardiac muscle with aging can be modulated by thyroxine. Circ Res 1987;60:238-245.

301. Gwathmey JK, Slawsky MT, Hajjar RJ, Briggs GM, Morgan JP. Role of intracellular calcium handling in force-interval relationships of human ventricular myocardium. J Clin Invest. 1990;85:1599-1613.

302. Dhalla NS, Wang X, Beamish RE. Intracellular calcium handling in normal and failing hearts. Exp Clin Cardiol. ,1996;1:7-20

303. Dhalla NS, Shao Q, Panagia V. Remodeling of cardiac membranes during the development of congestive heart failure. Heart Failure Rev 2.1998; 261-272,

304. Morgan JP, Erny RE, Allen PD, Grossman W, Gwathmey JK. Abnormal intracellular calcium handling, a major cause of systolic and diastolic dysfunction in ventricular myocardium from patients with heart failure. Circulation. 1990;81:III21-32.

305. Arai M, Matsui H, Periasamy M. Sarcoplasmic reticulum gene expression in cardiac hypertrophy and heart failure. Circ Res. 1994;74:555-564.

306. de la Bastie D, Levitsky D, Rappaport L, Mercadier JJ, Marotte F, Wisnewsky C, Brovkovich V, Schwartz K, Lompre AM. Function of the sarcoplasmic reticulum and expression of its Ca^{2+}-ATPase gene in pressure overload-induced cardiac hypertrophy in the rat. Circ Res. 1990;66:554-564.

307. Schwinger RH, Bohm M, Schmidt U, Karczewski P, Bavendiek U, Flesch M, Krause EG, Erdmann E. Unchanged protein levels of SERCA II and phospholamban but reduced Ca^{2+} uptake and Ca^{2+}-ATPase activity of cardiac sarcoplasmic reticulum from dilated cardiomyopathy patients compared with patients with nonfailing hearts. Circulation. 1995;92:3220-3228.

308. Mercadier JJ, Lompre AM, Duc P, Boheler KR, Fraysse JB, Wisnewsky C, Allen PD, Komajda M, Schwartz K. Altered sarcoplasmic reticulum Ca^{2+}-ATPase gene expression in the human ventricle during end-stage heart failure. J Clin Invest. 1990;85:305-309.

309. Meyer M, Schillinger W, Pieske B, Holubarsch C, Heilmann C, Posival H, Kuwajima G, Mikoshiba K, Just H, Hasenfuss G. Alterations of sarcoplasmic reticulum proteins in failing human dilated cardiomyopathy. Circulation. 1995;92:778-784.

310. Hasenfuss G, Reinecke H, Studer R, Meyer M, Pieske B, Holtz J, Holubarsch C, Posival H, Just H, Drexler H. Relation between myocardial function and expression of sarcoplasmic reticulum Ca^{2+}-ATPase in failing and nonfailing human myocardium. Circ Res. 1994;75:434-442.

311. Dhalla NS, Afzal N, Beamish RE, Naimark B, Takeda N, Nagano M. Pathophysiology of cardiac dysfunction in congestive heart failure. Can J Cardiol. 1993;10:873-887.

312. Litwin SE, Morgan JP. Captopril enhances intracellular calcium handling and beta-adrenergic responsiveness of myocardium from rats with postinfarction failure. Circ Res. 1992;71:797-807.

313. Afzal N, Dhalla NS. Sarcoplasmic reticular Ca^{2+} pump ATPase activity in congestive heart failure due to myocardial infarction. Can J Cardiol. 1996;12:1065-1073.

314. Iijima K, Geshi E, Nomizo A, Arata Y, Katagiri T. Alterations in sarcoplasmic reticulum and angiotensin II type 1 receptor gene expression after myocardial infarction in rats. Jpn Circ J. 1998;62:449-454.

315. Zhang XQ, Ng YC, Moore RL, Musch TI, Cheung JY. In situ SR function in postinfarction myocytes. J Appl Physiol. 1999;87:2143-2150.

316. Reffelmann T, Kloner RA. Transthoracic echocardiography in rats. Evalution of commonly used indices of left ventricular dimensions, contractile performance, and hypertrophy in a genetic model of hypertrophic heart failure (SHHF-Mcc-facp-Rats) in comparison with Wistar rats during aging. Basic Res Cardiol. 2003;98:275-284.

317. Solaro RJ, Pang DC, Briggs FN. The purification of cardiac myofibrils with Triton X-100. Biochim Biophys Acta. 1971;245:259-262.

318. Pierce GN, Dhalla NS. Mechanisms of the defect in cardiac myofibrillar function during diabetes. Am J Physiol. 1985;248:E170-e175.

319. Cummins P, Lambert SJ. Myosin transitions in the bovine and human heart. A developmental and anatomical study of heavy and light chain subunits in the atrium and ventricle. Circ Res. 1986;58:846-858.

320. Netticadan T, Temsah R, Osada M, Dhalla NS. Status of Ca^{2+}/calmodulin protein kinase phosphorylation of cardiac SR proteins in ischemia-reperfusion. Am J Physiol. 1999;277:C384-C391.

321. Netticadan T, Temsah RM, Kawabata K, Dhalla NS. Sarcoplasmic reticulum Ca^{2+}/Calmodulin-dependent protein kinase is altered in heart failure. Circ Res. 2000;86:596-605.

322. Netticadan T, Temsah RM, Kent A, Elimban V, Dhalla NS. Depressed levels of Ca^{2+}-cycling proteins may underlie sarcoplasmic reticulum dysfunction in the diabetic heart. Diabetes. 2001;50:2133-2138.

323. Temsah RM, Netticadan T, Chapman D, Takeda S, Mochizuki S, Dhalla NS. Alterations in sarcoplasmic reticulum function and gene expression in ischemic-reperfused rat heart. Am J Physiol. 1999;277:H584-H594.

324. Fabiato A. Computer programs for calculating total from specified free or free from specified total ionic concentrations in aqueous solutions containing multiple metals and ligands. Methods Enzymol. 1988;157:378-417.

325. Osada M, Netticadan T, Tamura K, Dhalla NS. Modification of ischemia-

reperfusion-induced changes in cardiac sarcoplasmic reticulum by preconditioning. Am J Physiol. 1998;274:H2025-H2034.

326. Guo X, Chapman D, Dhalla NS. Partial prevention of changes in SR gene expression in congestive heart failure due to myocardial infarction by enalapril or losartan. Mol Cell Biochem. 2003;254:163-172.

327. Takeishi Y, Bhagwat A, Ball NA, Kirkpatrick DL, Periasamy M, Walsh RA. Effect of angiotensin-converting enzyme inhibition on protein kinase C and SR proteins in heart failure. Am J Physiol. 1999;276:H53-H62.

328. Litwin SE, Katz SE, Morgan JP and Douglas PS, Serial echocardiographic assessment of left ventricular geometry and function after large myocardial infarction in the rat. Circulation 89.1994:345–354

329. Wang J, Liu X, Ren B, Rupp H, Takeda N, Dhalla NS, Modification of myosin gene expression by imidapril in failing heart due to myocardial infarction, J. Mol. Cell. Cardiol.2002;34:847– 857.

330. Wang J, Guo X, Dhalla NS. Modification of myosin protein and gene expression in failing hearts due to myocardial infarction by enalapril or losartan. Biochim Biophys Acta. 2004;1690:177-184.

331. Honig CR. Depression of myosin B by catecholamine analogs: mechanism and in vivo significance. Am J Physiol. 1968;214:357-364.

332. Harris DE, Work SS, Wright RK, Alpert NR, Warshaw DM. Smooth, cardiac and skeletal muscle myosin force and motion generation assessed by cross-bridge mechanical interactions in vitro. J Muscle Res Cell Motil. 1994;15:11-19.

333. Lowes BD, Minobe W, Abraham WT, Rizeq MN, Bohlmeyer TJ, Quaife RA,

Roden RL, Dutcher DL, Robertson AD, Voelkel NF, Badesch DB, Groves BM, Gilbert EM, Bristow MR. Changes in gene expression in the intact human heart. Downregulation of alpha-myosin heavy chain in hypertrophied, failing ventricular myocardium. J Clin Invest. 1997;100:2315-2324.

334. VanBuren P, Harris DE, Alpert NR, Warshaw DM. Cardiac V1 and V3 myosins differ in their hydrolytic and mechanical activities in vitro. Circ Res. 1995;77:439-444.

335. Imamura S, Matsuoka R, Hiratsuka E, Kimura M, Nakanishi T, Nishikawa T, Furutani Y, Takao A. Adaptational changes of MHC gene expression and isozyme transition in cardiac overloading. Am J Physiol. 1991;260:H73-H79.

336. Herron TJ, McDonald KS. Small amounts of alpha-myosin heavy chain isoform expression significantly increase power output of rat cardiac myocyte fragments. Circ Res. 2002;90:1150-1152.

337. Lowes BD, Gilbert EM, Abraham WT, Minobe WA, Larrabee P, Ferguson D, Wolfel EE, Lindenfeld J, Tsvetkova T, Robertson AD, Quaife RA, Bristow MR. Myocardial gene expression in dilated cardiomyopathy treated with beta-blocking agents. N Engl J Med. 2002;346:1357-1365.

338. Szado T, McLarnon M, Wang X, van Breemen C. Role of sarcoplasmic reticulum in regulation of tonic contraction of rabbit basilar artery. Am J Physiol Heart Circ Physiol. 2001;28:H1481-H489.

339. Fabiato A. Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. Am J Physiol. 1983;245:C1-C14.

340. Studer R, Reinecke H, Bilger J, Eschenhagen T, Bohm M, Hasenfuss G, Just H,

Holtz J, Drexler H. Gene expression of the cardiac Na^{+}– Ca^{2+}exchanger in end-stage human heart failure. Circ Res. 1994;75:443–453

341. Takahashi T, Allen PD and Izumo S. Expression of A-, B-, and C-type natriuretic peptide genes in failing and developing human ventricles. Correlation with expression of the Ca^{2+}-ATPase gene. Circ Res. 1992;71:9–17

342. Movsesian MA, Karimi M, Green K and Jones LR. Ca^{2+}-transporting ATPase, phospholamban, and calsequestrin levels in non-failing and failing human myocardium. Circulation. 1994;90:653–657

343. Schwinger RH, Munch G, Bolck B, Karczewski P, Krause EG and Erdmann E. Reduced Ca^{2+}-sensitivity of SERCA2a in failing human myocardium due to reduced serine16 phospholamban phosphorylation. J Mol Cell Cardiol.1999;31:479–491

344. Huang B, Wang S, Qin D, Boutjdir M and El Sherif N. Diminished basal phosphorylation level of phospholamban in the postinfarction remodeled rat ventricle: role of β-adrenergic pathway, Gi protein, phosphodiesterase, and phosphatases. Circ Res.1999;85:848–855

345. Wegener AD, Simmerman HK, Lindemann JP and Jones LR. Phospholamban phosphorylation in intact ventricles. Phosphorylation of serine16 and threonine17 in response to β-adrenergic stimulation. J Biol Chem 264 (1989), pp. 11468–11474 published erratum in J Biol Chem 1989;264:15738

346. Drago GA and Colyer J. Discrimination between two sites of phosphorylation on adjacent amino acids by phosphorylation site-specific antibodies to phospholamban. J Biol Chem.1994;269:25073–25077

347. MacDougall LK, Jones LR and Cohen P. Identification of the major protein phosphatases in mammalian cardiac muscle which dephosphorylate phospholamban. Eur J Biochem.1991;196:725–734

348. Tada M, Inui M, Yamada M, Kadoma M, Kuzuya T, Abe H, and Kakiuchi S. Effects of phospholamban phosphorylation catalyzed by adenocine 3',5' monophosphate- and calmodulin-dependent protein kinases on calcium transport ATPase of cardiac sarcoplasmic reticulum. J Mol Cell Cardiol 1983: 335-346

349. Schwinger RH, Bolck B, Munch G, Brixius K, Muller-Ehmsen J and Erdmann E. cAMP-dependent protein kinase A-stimulated sarcoplasmic reticulum function in heart failure. Ann NY Acad Sci.1998;853:240–250

350. Bristow MR, Ginsburg R, Minobe W, Cubicciotti RS, Sageman WS, Lurie K, Billingham ME, Harrison DC, Stinson EB. Decreased catecholamine sensitivity and β-adrenergic-receptor density in failing human hearts. New Engl J Med.1982;307:205–211

351. Neumann J, Schmitz W, Scholz H, von Meyerinck L, Doring V and Kalmar P. Increase in myocardial Gi-proteins in heart failure. Lancet.1988;2:936–937

352. Feldman AM, Ray PE, Silan CM, Mercer JA, Minobe W, Bristow MR. Selective gene expression on failing human heart: quantification of steady-state levels of messenger RNA in endomyocardial biopsies using the polymerase chain reaction. Circulation. 1991;83:1866–1872

353. Arai M, Alpert NR, MacLennan DH, Barton P, Periasamy M. Alterations in sarcoplasmic reticulum gene expression in human heart failure: a possible mechanism for alterations in systolic and diastolic properties of the failing

myocardium. Circ Res. 1992;72:463–469

354. Linck B, Bokník P, Eschenhagen T, Müller FU, Neumann J, Nose M, Jones LR, Schmitz W, Scholz H. Messenger RNA expression and immunological quantification of phospholamban and SR-Ca2+-ATPase in failing and nonfailing human hearts. Cardiovasc Res. 1996;31:625–632

355. Munch G, Bolch B, Hoischen S, Brixius K, Bloch W, Reuter H, Schwinger RH. Unchanged protein expression of sarcoplasmic reticulum Ca2+-ATPase, phospholamban, and calsequestrin in terminally failing human myocardium. J Mol Med. 1998;76:434–441

356. Igarashi M, Okuda T, Oh-i T, Koga M. Changes in plasma serotonin concentration and acceleration plethysmograms in patients with Raynaud's phenomenon after long-term treatment with a 5-HT2 receptor antagonist. J Dermatol. 2000;27:643-650

357. Rydzewski A, Urano T, Hachiya T, Kaneko H, Baba S, Takada Y, Takada A. The effect of a 5HT2 receptor antagonist sarpogrelate (MCI-9042) treatment on platelet function in Buerger's disease. Thromb Res. 1996;84:445-452

358. Fujita M, Mizuno K, Ho M, Tsukahara R, Miyamoto A, Miki O, Ishii K, Miwa K. Sarpogrelate treatment reduces restenosis after coronary stenting. Am Heart J. 2003;145:E16

359. Doggrell SA. The role of 5-HT on the cardiovascular and renal systems and the clinical potential of 5-HT modulation. Expert Opin Investig Drugs. 2003;12:805-823

360. Majid PA, Morris WM, Sole MJ. Hemodynamic and neurohumoral effects of

ketanserin, a 5-HT2 receptor antagonist in patients with congestive heart failure. Can J Cardiol. 1987;3:70-74.

361. Brune S, Schmidt T, Tebbe U, Kreuzer H. Influence of long-term treatment with ketanserin on blood pressure, pulmonary artery pressure, and cardiac output in patients with heart failure. Cardiovasc Drugs Ther. 1990;4 Suppl 1:85-87.

362. Grobecker H, Gessler I, Delius W, Dominiak P, Kees F. Effect of ketanserin on hemodynamics, plasma-catecholamine concentrations, and serotonin uptake by platelets in volunteers and patients with congestive heart failure. J Cardiovasc Pharmacol. 1985;7 Suppl 7:S102-S104.

363. Demoulin JC, Bertholet M, Soumagne D, David JL, Kulbertus HE. 5-HT2-receptor blockade in the treatment of heart failure. A preliminary study. Lancet. 1981;1:1186-1188.

364. Cobo C, Alcocer L, Chavez A. Effects of ketanserin on left ventricular hypertrophy in hypertensive patients. Cardiovasc Drugs Ther. 1990;4 Suppl 1:73-76.

365. Coto V, Cocozza M, Oliviero U, Lucariello A, Picano T, Castaldo B, Iovino V, Cacciatore L. Regression of left ventricular hypertrophy and systolic function in hypertensive patients during long-term treatment with ketanserin. Cardiovasc Drugs Ther. 1990;4 Suppl 1:77-80.

366. Nebigil CG, Maroteaux L. Functional consequence of serotonin/5-HT2B receptor signaling in heart: role of mitochondria in transition between hypertrophy and heart failure? Circulation. 2003;108:902-908

367. Feldman MD, Copelas L, Gwathmey JK, Phillips P, Warren SE, Schoen FJ,

Grossman W, Morgan JP. Deficient production of cyclic AMP: pharmacologic evidence of an important cause of contractile dysfunction in patients with end-stage heart failure. Circulation. 1987;75:331-339.

368. Wilmshurst PT, Walker JM, Fry CH, Mounsey JP, Twort CH, Williams BT, Davies MJ, Webb-Peploe MM. Inotropic and vasodilator effects of amrinone on isolated human tissue. Cardiovasc Res. 1984;18:302-309.

369. Packer M. Vasodilator and inotropic drugs for the treatment of chronic heart failure: distinguishing hype from hope. J Am Coll Cardiol. 1988;12:1299-1317

370. Packer M, Leier CV. Survival in congestive heart failure during treatment with drugs with positive inotropic actions. Circulation. 1987;75:IV55-63.

371. DiBianco R, Shabetai R, Kostuk W, Moran J, Schlant RC, Wright R. A comparison of oral milrinone, digoxin, and their combination in the treatment of patients with chronic heart failure. N Engl J Med. 1989;320:677-683.

372. Packer M, Medina N, Yushak M. Hemodynamic and clinical limitations of long-term inotropic therapy with amrinone in patients with severe chronic heart failure. Circulation. 1984;70:1038-1047

373. Alousi AA, Canter JM, Montenaro MJ, Fort DJ, and Ferrari RA. Cardiotonic activity of milrinone, a new and potent cardiac bipyridine, on the normal and failing heart of experimental animals. J Cardiovasc Pharmacol 5 1983: 792-803

374. Alousi AA, Stankus GP, Stuart JC, and Walton LH. Characterization of the cardiotonic effects of milrinone, a new and potent cardiac bipyridine, on isolated tissues from several animal species. J Cardiovasc Pharmacol 5 1983: 804-811

375. Pastelin G, Mendes R, Kabela E, and Farah A. The search for a digitalis

substitute II milrinone (Win 47203). Its action on the heart-lung preparation of the dog. Life Sci 33 1983: 1787-1796

376. Gaide MS, Fitterman WS, Wiggins JR, Myerburg RJ, Cameron JS, and Bassett AL. Amrinone relaxes potassium-induced contracture of failing right ventricular muscle of cats. J Cardiovasc Pharmacol 5 1983: 335-340

377. Scholz H. Pharmacological actions of various inotropic agents. Eur Heart J. 1983;4 Suppl A:161-172.

378. Tsien RW. Cyclic AMP and contractile activity in heart. Adv Cyclic Nucleotide Res. 1977;8:363-420.

379. Evans DB. Modulation of cAMP: mechanism for positive inotropic action. J Cardiovasc Pharmacol. 1986;8 Suppl 9:S22-S29.

380. Scholz H. Inotropic drugs and their mechanisms of action. J Am Coll Cardiol. 1984;4:389-397.

381. Minami N, Suzuki Y, Yamamoto M, Kihira H, Imai E, Wada H, Kimura Y, Ikeda Y, Shiku H, Nishikawa M. Inhibition of shear stress-induced platelet aggregation by cilostazol, a specific inhibitor of cGMP-inhibited phosphodiesterase, in vitro and ex vivo. Life Sci. 1997;61:PL 383-389

382. Yoshitomi Y, Kojima S, Sugi T, Yano M, Matsumoto Y, Kuramochi M. Antiplatelet treatment with cilostazol after stent implantation. Heart. 1998;80:393-396

383. Uretsky BF, Jessup M, Konstam MA, Dec GW, Leier CV, Benotti J, Murali S, Herrmann HC, Sandberg JA. Multicenter trial of oral enoximone in patients with moderate to moderately severe congestive heart failure. Lack of benefit

compared with placebo. Enoximone Multicenter Trial Group. Circulation. 1990;82:774-780.

384. Jain P, Brown EJ Jr, Langenback EG, Raeder E, Lillis O, Halpern J, Mannisi JA. Effects of milrinone on left ventricular remodeling after acute myocardial infarction. Circulation. 1991;84:796-804

385. Sweet CS, Ludden CT, Stabilito II, Emmert SE, Heyse JF. Beneficial effects of milrinone and enalapril on long-term survival of rats with healed myocardial infarction. Eur J Pharmacol. 1988;147:29-37

386. Ahnve S, Helmers C, Lundman T. QTc intervals at discharge after acute myocardial infarction and long-term prognosis. Acta Med Scand;208:55-60.

387. Ahnve S, Lundman T, Shoaleh-var M. The relationship between QT interval and ventricular arrhythmias in acute myocardial infarction. Acta Med Scand. 1978;204:17-19.

388. Lee JC, Downing SE. Cyclic AMP and the pathogenesis of myocardial injury. Res Commun Chem Pathol Pharmacol. 1980 Feb;27(2):305-18.

389. Podzuweit T, Lubbe WF, Opie LH. Cyclic adenosine monophosphate, ventricular fibrillation, and antiarrhythmic drugs. Lancet. 1976;1:341-342.

390. Scholz H, Meyer W. Phosphodiesterase-inhibiting properties of newer inotropic agents. Circulation. 1986;73:III99-108.