Membrane Remodeling in Heart Failure due to Myocardial Infarction in Rats

A Thesis

Presented to the

University of Manitoba

in Partial Fulfillment of the Requirements

for the Degree of Doctor of Philosophy

by

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FACULTY OF GRADUATE STUDIES

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MEMBRANE REMODELING IN HEART FAILURE DUE TO MYOCARDIAL INFARCTION IN RATS

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A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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This thesis is dedicated to

My motherland China

and my family

ABSTRACT

By employing a rat model of heart failure following coronary occlusion, we have tested the hypothesis that beneficial effects of imidapril (IMP), a long acting angiotensin converting enzyme inhibitor, on heart failure are associated with prevention of Ca^{2+}-handling abnormalities in cardiomyocytes. In this study, IMP (1 mg/kg, daily) was given orally for 4 weeks starting 3 weeks after coronary occlusion (myocardial infarction) or sham operation. Untreated sham control or infarcted rats were given saline under similar conditions. Occlusion of the coronary artery in rats for 7 weeks was found to result in cardiac hypertrophy, elevation in left ventricular end diastolic pressure (LVEDP) and depression in both rates of contraction (+dP/dt) and relaxation (-dP/dt) without any changes in the heart rate or left ventricular systolic pressure. Treatment of infarcted animals with IMP prevented these changes in heart function fully or partially without significantly affecting the scar weight or the increase in left ventricular weight. The depression in ATP-induced increases in left ventricular developed pressure, +dP/dt and -dP/dt in the infarcted animals was also prevented by IMP treatment. Although basal $[Ca^{2+}]_i$ in cardiomyocytes was not altered by myocardial infarction, both ATP-induced and KCl-induced increases in $[Ca^{2+}]_i$ were depressed in the infarcted animals and these changes were prevented by IMP treatment. Neither the maximal number nor the affinity of ATP-receptors in sarcolemmal membranes were affected in infarcted animals. Treatment of sham control animals with IMP did not exert any effect on cardiac performance, ATP-induced changes in heart function, ATP- or KCl-induced increase in $[Ca^{2+}]_i$ in cardiomyocytes, and ATP-receptors. These results suggest that cardiac dysfunction subsequent to myocardial infarction is not associated with changes in $[Ca^{2+}]_i$; however, the loss of modulatory effect of ATP in the failing heart may

be related to Ca^{2+}-handling abnormalities in cardiomyocytes.

In view of the crucial role played by sarcolemma (SL) in the control of Ca^{2+}-movements in cardiomyocytes, we examined the status of SL remodeling in the failing hearts subsequent to myocardial infarction. The SL Na^+-K^+ ATPase activity was depressed in the failing left ventricles and this was accompanied by a decrease in the α_1-, α_2- and β_1-isoform and an increase in α_3-isoform contents in the SL membrane. A depression in mRNA levels for α_1-, α_2- and β_1-isoforms and an increase in α_3-isoform of Na^+-K^+ ATPase were also observed in the failing hearts. These changes in Na^+-K^+ ATPase activity and protein content as well as gene expression of different Na^+-K^+ ATPase isoforms were partially or fully prevented by treatment of infarcted animals with IMP. The depression in Na^+-dependent Ca^{2+}-uptake activity and protein content as well as mRNA levels for Na^+-Ca^{2+} exchange in the failing hearts was also fully or partially prevented by IMP treatment. The activities, protein contents and gene expression for Na^+-K^+ ATPase and Na^+-Ca^{2+} exchanger were not affected in the sham control animals upon treatment with IMP. These results suggest that remodeling of SL membrane in failing heart is associated with changes in the SL activities and protein contents of Na^+-Ca^{2+} exchange and Na^+-K^+ ATPase isoforms. Furthermore, the beneficial effects of IMP treatment in heart failure due to myocardial infarction may be due to the ability of this drug to prevent remodeling of SL membrane.

By virtue of its ability to release and accumulate Ca^{2+}, the sarcoplasmic reticulum (SR) is known to be involved in the processes of cardiac contraction and relaxation, respectively. Accordingly, we investigated the status of Ca^{2+}-pump and Ca^{2+}-release channels in SR membranes from the failing left ventricles subsequent to myocardial infarction. The activities of ATP-dependent Ca^{2+}-uptake and Ca^{2+}-stimulated ATPase as well

as protein contents of Ca^{2+}-pump ATPase in SR membranes were depressed in the failing hearts. Likewise, Ca^{2+}-release channels, as monitored by ^{3}H-ryanodine binding, and protein content were decreased in SR from failing hearts. These changes in the failing hearts were also associated with a depression in mRNA level for Ca^{2+}-pump in the failing hearts. Although gene expression for phospholamban, which regulates the SR Ca^{2+}-pump activity, was depressed in the failing heart, mRNA levels for calsequestrin, which binds Ca^{2+} in the lumen, was unaltered. The observed alterations in the activities, protein content and gene expression for Ca^{2+}-pump was fully or partially prevented by treatment of infarcted animals with IMP; this treatment had no effect on these parameters in sham control animals. These results suggest that the observed changes in SR Ca^{2+}-pump and Na^{+}-Ca^{2+} exchanger in the failing hearts due to myocardial infarction may be a consequence of altered gene expression. The beneficial effect of IMP treatment in the failing heart may be due to the ability of this agent to prevent remodeling of SR membrane by modification of the altered gene expression.

From the results presented in this study, it is evident that the improvement of heart failure subsequent to myocardial infarction by imidapril treatment is seen at the level of cardiac performance in the intact animal, cardiocyte preparations, biochemical activities of isolated membranes and cardiac gene expression. It is concluded that the beneficial effects of imidapril on heart function are associated with the ability of this drug to prevent the remodeling of cardiac membranes, improve Ca^{2+}-handling abnormalities in cardiomyocytes and modify gene expression specific for membrane proteins in the failing hearts.

ACKNOWLEDGMENTS

I wish to thank my supervisor, Dr. Naranjan S. Dhalla for giving me the opportunity to work with him. His continued support, guidance and encouragement led me through the course of this study. His understanding of my cultural and scientific background had an invaluable positive influence on me and he will inspire my future professional life. I would also like to take this opportunity to thank the members of my graduate committee: Dr. P.K. Singal, Dr. V. Panagia and Dr. P. Zahradka for their guidance, advice and time during the pursuit of my Ph.D degree. As well, I would like to thank the other faculty members of the Institute of Cardiovascular Sciences at the St. Boniface General Hospital Research Centre, who have been most approachable and helpful throughout my student career in this department.

I am grateful to all people in the Experimental Cardiology laboratory (past and present) for their scientific and personal interaction and many fond memories: Vijayan Elimban, Ken Dhalla, Donald Chapman, Kiminori Kato, Adriana Condello, Xi Wang, Xueliang Liu, Jing Wei Wang, Subburaj Kannan, Sorin Musat-Marcu, Sujata Persad, Satoshi Takeda, Kannu Shah, Rajat Sethi and Nasir Afzal. Special thanks are due to Susan Zettler, Mary Brown and Florence Willerton for their continued support. I would also like to recognize Dr. Randy Aitken, Karan Moore, Diana Saunders and Nicole Rebeck in Animal Holding of St. Boniface General Hospital, without their support and help, I would not have been as successful. The staff of the Building Security deserve thanks as well for opening the doors for the laboratory in evenings and on weekends.

I extend my thanks to Dr. N. Stephens who gave me the idea of coming to Winnipeg and supporting me from the beginning to the end of my studies. Thanks are also due to Dr. Arnold Naimark and Dr. Barbara Naimark, and Dr. & Mrs. Dick Lim for making me feel as a part of their families. I also appreciated the help of two other Canadian families: the Besants and the Fehrs, who took care of my son and put my mind at ease to devote more energy to my work.

I am highly indebted to my parents, my in-laws and my brother for their long distance moral support, understanding and encouragement to achieve my best. Their advice and help have been immeasurable throughout my life. I owe many special thanks to Bin Ren, my loving husband, without his strong support, I would not have achieved my professional goal. I am also very grateful to my loving son Ke Ren for being a terrific, intelligent, humourous, understanding and cooperative child.

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I. LITERATURE REVIEW

1. Ca^{2+} transport in cardiac membrane

It has now become clear that Ca^{2+} is a critical physiological regulator of contractile and metabolic processes in cardiac muscle (1-3). Both sarcolemma (SL) and sarcoplasmic reticulum (SR) are intimately involved in the regulation of intracellular Ca^{2+} in cardiomyocytes (2, 3). Although Ca^{2+} also accumulates in mitochondria and nucleus, the physiological significance of these Ca^{2+}-transport systems is poorly understood. It should be noted that Ca^{2+}-channels and Ca^{2+}-pump present in SL are concerned with Ca^{2+}-influx and Ca^{2+}-efflux, respectively. On the other hand, Na^{+}-Ca^{2+} exchanger is considered to be involved in both Ca^{2+}-influx and Ca^{2+}-efflux processes. Furthermore, Ca^{2+} from the cytoplasm is taken up by an energy-dependent mechanism (Ca^{2+}-pump ATPase) present in the SR membrane and released through Ca^{2+}-induced Ca^{2+}-release channels located in this membrane system. In this review therefore, it is our intention to highlight some of the new information regarding the status of Ca^{2+}-transport mechanisms in both SL and SR membranes of the healthy and failing hearts.

A. SL Ca^{2+} transport mechanisms

Evaluation of physiological and biochemical data has shown that SL Ca^{2+} transport is effected by several mechanisms including Na^{+}-Ca^{2+} exchanger, Ca^{2+}-pump, Na^{+}-pump and Ca^{2+} channels. Accordingly, it is planned to discuss these systems with respect to their functional significance.

(i) SL Na^+-Ca^{2+} exchanger

In comparison to SR which regulates about 80% of the intracellular Ca^{2+} in cardiomyocytes, the extent of contribution by SL Na^+-Ca^{2+} exchanger varies between 10-20% (4, 5). The Na^+-Ca^{2+} exchanger, which was first identified in 1968 to be present in the cardiac muscle (6), is a major pathway for transmembrane Ca^{2+} fluxes in the SL membrane. It is known to play a significant role in the excitation-contraction coupling process in cardiac muscle and is a carrier-mediated transport process in which the movement of calcium ions across the membrane is obligatorily coupled to the movement of Na^+ ions in the opposite direction. In cardiac cells, the stoichiometry of SL Na^+-Ca^{2+} exchanger is 3 Na^+ per 1 Ca^{2+} (7). The reaction of Na^+-Ca^{2+} exchanger is that of a ping-pong model where the concentration of unloaded carrier on one side of the membrane depends upon the rate at which the carrier is translocated across the membrane from the opposite side. It has high capacity (V_{max} = 30-40 nmol) and low affinity for calcium (K_a > 10 μM). The Na^+-Ca^{2+} exchanger protein has been purified, cloned and sequenced in canine cardiac SL by Philipson and his colleagues (8, 9). There are three Na^+-Ca^{2+} exchanger genes in mammals (10-12). The mammalian heart Na^+-Ca^{2+} exchanger has 11 putative transmembrane domains and a long intracellular loop. The cytosolic side of the exchanger contains inhibitory peptide domain, Ca^{2+} binding domain, alternative splicing site and potential PKA phosphorylation site. In the heart, Na^+-Ca^{2+} exchanger is thought to function primarily as a mechanism for pumping Ca^{2+} out of the cell; however, it is also known to promote the net entry of Ca^{2+} into the cell under certain circumstances such as membrane depolarization.

The distribution of Na^+-Ca^{2+} exchanger is plentiful in the SL membrane. There are approximately 250-400 Na^+-Ca^{2+} exchanger proteins per square micron of the membrane (13, 14); these are distributed in T-tubules, intercalated disc area and the area adjacent to gap junctions in addition to the peripheral SL (15). Existence of a Ca^{2+} regulatory site in Na^+-Ca^{2+} exchanger was first observed by DiPolo (16) and in fact the regulation of Na^+-Ca^{2+} exchanger has now been examined from both subcellular and molecular aspects. The Na^+-Ca^{2+} exchanger is regulated by Ca^{2+}, ATP, phosphorylation, pH and lipids (17-20); Ca^{2+} regulates both outward and inward exchanger currents. Phosphorylation of Na^+-Ca^{2+} exchanger was reported to be tissue specific; Na^+-Ca^{2+} exchanger activity was increased by protein kinase C (PKC) stimulation in smooth muscle (21) and decreased by protein kinase A (PKA) stimulation in chromaffin cells, epithelial cells as well as cardiomyocytes (22, 23). The molecular regulation of Na^+-Ca^{2+} exchanger is referred to as Na^+-dependent inactivation which means that upon the application of Na^+ at the intracellular surface, three Na^+ ions will bind at the intracellular transport sites and the exchanger will enter into an inactivated state (23). The purified protein appears as two bands on sodium dodecyl sulfate (SDS) gel at 120 and 160 Kd. In some preparations, there is also a band at 70 Kd; this appears to be a proteolytic degradation product of the higher molecular weight. The sequence of the cloned exchanger exhibits a 32-amino-acid N-terminal segment which has the characteristics of a signal sequence of the purified protein.

(ii) Ca^{2+} channels

Calcium channels are intrinsic membrane glycoproteins that participate in the

regulation of transmembrane ion flow and cellular function in the heart (24). Ca^{2+} channels in the myocardium provide the major pathway for Ca^{2+} entry into the cells as these open in response to depolarization of the surface membrane. These channels allow Ca^{2+} to enter into the cells to initiate the cellular functions such as excitability, contraction and secretion (25). At least two types of calcium channels (L-type and T-type) exist in cardiac SL membrane in different species (26-28). Differences between L- and T-type calcium channels were well described by Balke et al (29). The most important and abundant plasma membrane calcium channel is the L-type channel which plays a crucial role in the process of coupling excitation with contraction. The L-type calcium channel is made up of five subunits, namely α_1, α_2, β, δ and γ (30); these subunits may function individually or coeffect the function of the channel, in which the α_1-subunit plays an important role (31, 32). The opening of the calcium channel can be stimulated by Bay K, a Ca^{2+} channel agonist, or blocked by antagonists such as dihydropyridines, phenylalkylamines and benzothiazepines (30). The L-type calcium channels are regulated by sympathetic stimulus and hormonal factors (33), α- and β-adrenergic signaling pathways (34) as well as cGMP dependent protein kinase (35, 36). The T-type Ca^{2+} channel in heart is similar to the L-type Ca^{2+} channel because it is opened by depolarization; however, both T- and L-type Ca^{2+} channels differ in several ways, including opening and closing time, distribution as well as importance in the excitation and relaxation processes. The diversity of the Ca^{2+} channels has been reviewed recently (37).

(iii) Ca^{2+}-pump ATPase

The presence of Ca^{2+} pump in the plasma membrane was first suggested by Dunham et al (38) and confirmed by Schatzmann (39). The function of the plasma SL Ca^{2+}-pump for Ca^{2+}-efflux is less important than that of the SL Na^{+}-Ca^{2+} exchanger in the heart. In fact, SL Ca^{2+}-pump has been shown to play a minor role in transporting Ca^{2+} in comparison to other Ca^{2+}-handling mechanisms; its properties were described by Carafoli (3). Four genes of plasma membrane Ca^{2+}-pump have been reported (PMCA1, PMCA2, PMCA3 and PMCA4), but only PMCA1 and PMCA4 exist in the heart (40). The plasma membrane Ca^{2+}-pump has a molecular weight of about 120-130 KD and consists of 10 transmembrane α-helices which possess ATP catalytic domain as well as the regulating domain. The activity of SL Ca^{2+}-pump is regulated by a multiplicity of mechanisms, including calmodulin (41), acidic phospholipids (42) and kinase-mediated phosphorylations (43). Recently the possibility of regulating the SL Ca^{2+}-pump by G-proteins was suggested by some investigators (40).

(iv) SL Na^{+}-K^{+} ATPase

Na^{+}-K^{+} ATPase is a ubiquitous transmembrane enzyme which transport of Na^{+} ions out of the cell and moves K^{+} ions into the cell by utilizing ATP as the driving force (44). This enzyme is a member of the P-type ATPase family, which is found in the cells of all higher eukaryotes. The Na^{+}-K^{+} ATPase maintains the electro-chemical gradient across the cell membrane and is coupled to other transport mechanisms which are important for cell homeostasis and specialized function (45). The characteristic feature of the Na^{+}-K^{+} ATPase is that it is activated by a combined effect of Na^{+} on cytoplasmic sites and of K^{+} on

extracellular sites in the presence of ATP and Mg^{2+}. The cytoplasmic K^+ inhibits the activity of Na^+-K^+ ATPase by competing for the binding of cytoplasmic Na^+ whereas the extracellular Na^+ inhibits by competing for the binding of extracellular K^+. An important finding that cardiac glycosides inhibit the active transport in red blood cells was made by Schatzmann (46). The Na^+ and K^+ affect the association rate constant for glycoside binding, in which Na^+ increases whereas K^+ decreases the association rate of glycoside binding. In the heart, Na^+-K^+ ATPase participates in repolarization of the membrane during phase 4 of the action potential. The specific inhibition of Na^+-K^+ ATPase by cardiac glycosides leads to a positive inotropic effect by increasing the intracellular Na^+ concentration which in turn results in the elevation of the intracellular concentration of Ca^{2+} and increase in the force of contraction of the heart.

Na^+-K^+ ATPase is now known to belong to a multigene family. The enzyme is composed of two subunits, a large catalytic α-subunit (Mr 112,000) and a smaller β-subunit (Mr 35,000-55,000), which is responsible for the maturation and transport of the enzyme (47); there are multiple isoforms of each subunit (α_1, α_2, α_3, β_1, β_2 and β_3). The amino acid sequence of the α- as well as β-unit has been determined from cDNA. The α-chain consists of 1,016 amino acids and has 8 hydrophobic regions. The N-terminal hydrophilic region which consists of 92 amino acids, is on the cytoplasmic side and is followed by 4 transmembrane segments with a cytoplasm loop between segments 2 and 3 of about 145 amino acids. The ATP binding site is located between segments 4 and 5. The β-chain consists of 302 amino acids with one transmembrane segment located near the cytoplasmic N-terminal and the major, hydrophilic part of the molecule on the extracellular side. Three

distinct isoforms of the α-subunit (α_1, α_2 and α_3), encoded by three distinct genes, have been identified by molecular genetic and immunological techniques (48, 49) and have been shown to be both hormonally and developmentally regulated (50). In rat cardiac tissue, the α_1-mRNA is the major α-isoform transcript (~70-75% of total α-mRNA abundance) expressed at all developmental stages. On the other hand, the α_2- and α_3-isoform gene transcripts are present in minor quantities (~25-30% of total α-mRNA abundance) and are developmentally regulated. The α_3-isoform is expressed primarily in fetal and neonatal heart. After birth, the α_3-isoform declines to a negligible level and is replaced by α_2-isoform (51). The α-isoforms differ primarily in their affinity for ouabain and other cardiac glycosides, with α_1 exhibiting a low affinity whereas α_2 and α_3 exhibit an approximately 1000-fold higher affinity (48). Different isoforms of the Na^+-K^+ ATPase are expressed in different cell types in which they contribute towards specialized properties.

The β-subunit forms a complex with the α-subunit and exists in a one to one ratio with α-subunit. Although the exact function of β-subunit is still not clearly defined, it has been recognized that the β-subunit plays a critical role in the assembly and integration of the mature Na^+-K^+ ATPase to plasma membrane (52, 53). Using the Xenopus oocyte as an expression system, Noguchi et al (54) were the first to demonstrate that an injection of both α- and β-cDNA is needed to express functional activity of the Na^+-K^+ pump at the plasma membrane. Kawamura and Nagano (55) as well as Kirley (56) have reported that reduction of disulfide bonds in the β-subunit results in a complete loss of enzyme activity. The β-subunit of Na^+-K^+ ATPase has also been shown to prevent trypsin-mediated degradation

of the α-subunit (57). It should be pointed out that the Na^+-K^+ ATPase isoform distribution is tissue and species specific. All three α-isoforms are detected in human heart (58), whereas only α_1- and α_2-isoforms are expressed in adult rat heart; α_1- and α_3-isoforms are present in ferret and dog heart (59, 60). The relative distribution of three α-isoforms varies with the cell types, there are 30-40% in rat left ventricle and <10% in rat atrium (58). The results from the Northern blot analysis showed that β-subunit is present in equal amounts in the left ventricle, right ventricle and atria of the heart. In contrast, α_1-subunit mRNA is present in slightly greater amount in the atria and α_2-mRNA is slightly more abundant in the ventricles (61).

B. SR Ca^{2+} transport mechanisms

Ultrastructural examination of mammalian myocardium has shown that SR is composed of at least three different structures: (a) the longitudinal SR which is formed by a network of tubules surrounding the myofibrils, (b) the junctional SR composed of cisternae which is continuous with the SR network at one end and in contact with the T-tubules at the other end to form triads, (c) a specialized non-junctional SR called tubular SR which represents extensions of the longitudinal SR not opposed to the SL or T-tubules but rather confined to the I-band of the sarcomere. SR membranes contain several proteins which play important roles in the accumulation, binding, release and regulation of intracellular Ca^{2+}.

(i) SR Ca^{2+}-pump ATPase

The Ca^{2+} uptake activity of SR is mediated by a Ca^{2+}-pump protein, SERCA, which is a single large transmembrane polypeptide of about 110 kD and represents about 40% of

the total protein in the longitudinal SR (62). This enzyme catalyzes Ca^{2+}-transport into the lumen of SR by an active process requiring ATP hydrolysis. Five distinct Ca^{2+}-pump ATPase isoforms encoded by three different genes (SERCA1, SERCA2, and SERCA3) have been identified: the adult fast-twitch skeletal muscle isoform (SERCA1a), its alternatively spliced neonatal isoform (SERCA1b), the cardiac/slow-twitch skeletal muscle isoform (SERCA2a), its alternatively spliced smooth muscle/nonmuscle isoform (SERCA2b), and an isoform expressed in a broad variety of muscle and non-muscle tissues (SERCA3). The cardiac muscle primarily expresses the SERCA2 isoform both in the atrium and ventricle (63). The proposed structure of the Ca^{2+}-pump ATPase has been based on the amino acid sequence and X-ray diffraction (64). According to this model, the Ca^{2+}-pump ATPase is formed by 10 transmembrane α-helices (M1-M10) separated by extramembranous loops which form a cytosolic domain. The functional differences between these various Ca^{2+}-pump ATPase isoforms have been investigated by transfecting different SERCA cDNA isoforms into COS-1 cells. The affinity for Ca^{2+} of SERCA2b was higher than SERCA2a; this isoform transported Ca^{2+} more slowly and hydrolyzed ATP with a lower turnover rate. It should be mentioned that SR Ca^{2+}-pump ATPase is different from the plasma Ca^{2+}-pump ATPase because the subunit size of SR Ca^{2+}-pump ATPase is 100-115 kD, while that present in SL is of 120-130 kD. Furthermore, SR Ca^{2+}-pump ATPase is regulated by phosphorylation of phospholamban whereas SL Ca^{2+}-pump ATPase is regulated by calmodulin (64, 65).

(ii) SR regulatory protein

The function of SR Ca^{2+}-pump is modulated by phospholamban which is an intrinsic

protein of five identical monomers with molecular weight of 6 kD (66). Each monomer is an amphipathic peptide consisting of 52 amino acid residues and is localized with SERCA2 in the longitudinal SR membrane. Several experiments have indicated that phospholamban inhibits SR Ca^{2+}-pump ATPase through a direct protein-protein interaction (66, 67). In vivo, phospholamban is phosphorylated (a) at Ser 16 by cAMP-dependent PKA, (b) at Thr 17 by Ca^{2+}-calmodulin-dependent protein kinase, and (c) at Ser 10 by Ca^{2+}-phospholipid-dependent PKC. In isolated cardiac SR vesicles, phospholamban is phosphorylated by cAMP-dependent, Ca^{2+}-calmodulin-dependent and Ca^{2+}-phospholipid-dependent protein kinases and results in an increase in the Ca^{2+}-pump ATPase activity and the rate of Ca^{2+}-uptake activity; such an effect can be seen to increase the rate of myocardial relaxation (68). In its unphosphorylated form, phospholamban inhibits the SR Ca^{2+}-pump ATPase activity by interacting with the enzyme and decreasing its affinity for Ca^{2+}. The phospholamban binding domain of the ATPase has been mapped downstream from the phosphorylation domain (69). Phosphorylation of phospholamban and the regulatory effects of three protein kinases on SR Ca^{2+}-pump can be reversed through dephosphorylation by an endogenous phosphatase (70).

(iii) SR Ca^{2+} binding proteins

Ca^{2+} binding proteins such as calsequestrin, calreticulin and sarcolumenin are very important for maintaining the concentration of cytosolic Ca^{2+}. Calsequestrin is a major Ca^{2+} storing protein located in the SR terminal cisternae (junctional and corbular SR) of muscle cells (71, 72). It serves as Ca^{2+} buffer and thus lowers the concentration of Ca^{2+} in the lumen of SR. The properties and the structure of calsequestrin was reviewed by Milner et al (73).

It is pointed out that calsequestrin is an acidic protein of about 60 kD and has some characteristic Ca^{2+} binding properties (74). Cardiac calsequestrin is different from the skeletal calsequestrin. Although it can be phosphorylated by casein kinase II, the function of the calsequestrin phosphorylation is as yet unknown. During skeletal muscle differentiation, the major Ca^{2+} storing protein switches from calreticulin to calsequestrin. Experiments by Inanaka-Yoshida et al (75) indicated that the calreticulin expression is down-regulated during cardiac differentiation and up-regulated during de-differentiation; the maturation of SR involves the organization of calsequestrin-positive structure after birth. Calsequestrin is a high-capacity moderate-affinity Ca^{2+} binding protein, which is responsible for the Ca^{2+} storage capacity of SR in striated muscles whereas other Ca^{2+} binding proteins such as calreticulin and sarcolumenin also play some role in buffering the free Ca^{2+} (75, 76). Sarcolumenin is a 160 kD glycoprotein with a low affinity but high capacity for Ca^{2+} binding whereas calreticulin is a minor Ca^{2+} binding protein in the cardiac SR (76).

(iv) SR Ca^{2+} release channel

The contraction of cardiac myocytes is triggered by Ca^{2+} release channel, also referred to as the ryanodine receptor (RYR). Two distinct isoforms of Ca^{2+} release channel, RYR1 and RYR2, have been described by cDNA cloning but only the RYR2 isoform is expressed in cardiac tissues. It is pointed out that ryanodine is a highly toxic plant alkaloid which exerts complex effects on cardiac and skeletal muscles, and uncouples the process of excitation from contraction. RYR has been identified as a protein with a molecular weight of about 400,000-450,000 (77, 78) and is located in the triadic structures where junctional

SR and adjacent portions of the T-tubule system of the SL are joined by foot processes. It is now believed that RYR corresponds both to the SR Ca^{2+} release channel and the "foot" process observed in electron micrographs at junctions between the SL and SR (79). This protein is important because of its response to a surface membrane action potential in the release of Ca^{2+} results in excitation-contraction coupling. Ryanodine can either cause contracture or a decline in contractile force; nanomolar to micromolar concentrations cause the formation of an open subconductance channel state, whereas at concentrations above 100 micromolar, ryanodine completely closes the Ca^{2+}-release channel. The molecular weight of ryanodine receptor is 560 kD; there has three isoforms of ryanodine receptor; skeletal muscle (RYR1) cardiac muscle (RYR2) and brain (RYR3). In single channel measurements, calmodulin was shown to inhibit the Ca^{2+}-release channel by reducing conductance. In vitro phosphorylation of a serine residue (Ser2809) by a calmodulin kinase has been reported to activate the calmodulin-inhibited RYR in the isolated cardiac muscle.

From the foregoing discussion it is evident that Ca^{2+} plays an important role in heart function and its regulation by both SL and SR membranes is crucial for cardiac contraction and relaxation processes. Depolarization of cardiac cell opens Ca^{2+}-channels in the SL membrane to permit a small quantity of Ca^{2+}; this cation may also enter the cell directly through the SL Na^{+}-Ca^{2+} exchanger. This depolarization-dependent Ca^{2+}-influx releases additional Ca^{2+} via Ca^{2+}-release channels in the SR membrane and thus induces contraction of actin and myosin filaments by its interaction with troponin-tropomyosin complex. The entry of Ca^{2+} in the cell is also modulated by SL Na^{+}-K^{+} ATPase where Ca^{2+} is exchanged

for Na^+ under conditions associated with the inhibition of Na^+-K^+ ATPase. On the other hand, the cytoplasmic concentration of Ca^{2+} is lowered by the activation of SR Ca^{2+}-pump ATPase mainly and SL Ca^{2+}-pump ATPase to some extent; Ca^{2+} is also removed from the cytoplasm by SL Na^+-Ca^{2+} exchanger for initiating relaxation of the contractile apparatus. The transport of Ca^{2+} in the SR tubules is markedly influenced by the presence of phospholamban in the SR membrane whereas Ca^{2+} is stored in the SR lumen by binding mainly with calsequestrin. Thus defects in the SL and/or SR membrane can result in Ca^{2+}-handling abnormalities in cardiomyocytes and cardiac dysfunction in the failing heart. Accordingly it is proposed that heart failure may be associated with remodeling of SL and SR membranes and any intervention which is known to exert a beneficial effect on the failing heart can be considered to prevent the remodeling of cardiac membrane. Such a mechanism of drug action may occur at the gene expression level where changes in mRNA abundance for specific membrane proteins are attenuated in the failing heart upon drug treatment. Further discussion in this review is therefore focussed on Ca^{2+}-handling abnormalities in cardiac dysfunction in general and heart failure in particular. Since myocardial infarction is a leading cause of heart failure, it is intended to discuss the overall pathophysiology of heart failure subsequent to myocardial infarction.

2. Pathophysiology of heart failure due to infarction

Congestive heart failure occurs when the heart is unable to pump sufficient blood to the body tissues to meet ordinary metabolic demands. Approximately 4 million Americans

suffer from heart failure; about 400,000 patients develop heart failure each year. About 50% of patients with heart failure die in the first year whereas the remaining 50% will die within 5 years. The causes of heart failure are classified into three groups: (a) mechanical abnormalities such as increased pressure load, volume load, obstruction to ventricular filling and endocardial or myocardial restriction, (b) myocardial abnormalities including loss of myocyte, cardiomyopathy, neuromuscular disorders, myocarditis, ischemia and inflammation, (c) rhythm abnormalities such as conduction disturbances, fibrillation and chronic tachycardia. It is pointed out that heart failure is associated with several changes in the myocardium: (a) mechanical alterations such as decrease in force development, decrease in the rate of force development, decrease in velocity of shortening and decrease in the rate of left ventricular relaxation, (b) receptors and signal transduction alterations include down-regulation and uncoupling of β_1-adrenergic receptors in heart failure, uncoupling of β_2-adrenergic receptors without any changes in their density, increase in α_1-adrenergic receptor density, decrease in vasopressin receptor density with increase in affinity, increase in G_i-proteins resulting in inhibition of adenylyl cyclase, decrease in cAMP production due to adenylyl cyclase inhibition, and (c) molecular alterations include switch from α-myosin heavy chain to β-myosin heavy chain, up-regulation of β-MHC gene expression, changes in α-actin isoform expression, up-regulation of α-skeletal isoactin gene expression and up-regulation of troponin T_1 and troponin T_2 expression. Subcellular remodeling in the failing heart is also reflected by changes in the expression of SR and SL proteins.

The varying etiology, uncertainty in defining the time of onset, and various cardiac

and peripheral alterations make it difficult to investigate the pathophysiology of congestive heart failure. The identification of appropriate experimental models of congestive heart failure also adds to the complexity of the problem. It should be pointed out that models of congestive heart failure are usually classified into four categories, namely pressure overload, volume overload, myocardial infarction and cardiomyopathy, which are induced by a variety of interventions (Table 1). Models using pressure overload are more suitable for the study of left ventricular hypertrophy which may or may not be associated with congestive heart failure. On the other hand, models using volume overload are involved volume infusion, or creation of shunts; these models create high cardiac output instead of reduced cardiac output commonly seen in human patients with congestive heart failure. Experimental models induced by myocardial cell damage or loss of cardiac tissue may mimic human heart failure, but the accompanying structural changes in cell types other than cardiomyocytes may complicate studies involving cardiac tissue. An irreversible injury following coronary artery occlusion results in a loss of cardiomyocyte units which are then replaced by scar tissue, mainly comprising connective tissue and other extracellular matrix material. The remaining viable myocardium eventually undergoes hypertrophy under the imposed need for compensatory work and then over a certain period it begins to fail to function adequately. It is indeed a challenge for experimental cardiologists to determine what leads to the transition from compensated cardiac hypertrophy to heart failure.

It is now becoming clear that several changes occur in both the infarcted and noninfarcted left ventricle early after myocardial infarction leading to progressive chamber enlargement (80). This remodeling process is determined by three factors, namely infarct

Table 1: Classification of experimental models of heart failure

Heart failure due to	Procedures and interventions employed for inducing heart failure
1. Pressure Overload	(a) Pulmonary artery banding (b) Aortic banding (c) Aortic valve constriction
2. Volume Overload	(a) Aorto-venal caval fistula (b) Aortic valve incompetence (c) Atrial septiac defect
3. Myocardial Infarction	(a) Coronary artery thrombosis (b) Coronary artery ligation (c) Coronary artery atherosclerosis
4. Cardiomyopathy	(a) Genetically-induced (b) Hormone- and drug-induced (c) Chronic pacing-induced

size, infarct healing and ventricular wall stress (81). The sudden occlusion of a large subepicardial coronary artery leads to a loss of the myocardium within minutes and reduces the performance of the ventricular pump in proportion to the amount of ischemic tissue (82). Structural and topographical remodeling of the left ventricle has long been recognized to develop following acute myocardial infarction. This remodeling is progressive in nature in that it develops over a period of months or even years after the acute event. The factors that dictate the rate at which this process develops are not clear but are likely related to the extent of loss of viable myocardium. A larger infarction is likely to elicit a faster progression of the left ventricular remodeling in comparison to a smaller infarction (83-86). It should be noted that the term "ventricular remodeling" includes several structural and topographical adaptations and/or maladaptations in response to myocardial injury. Globally, these changes include left ventricular chamber dilation and increased chamber specificity (87). At the cellular level, alterations such as an increase in myocyte size and accumulation of collagen in the interstitium as well as the cardiac membrane occur in both the myocyte and non-myocyte compartments.

3. Alterations in gene expression for Ca^{2+}-related proteins in SL and SR membranes

In view of the crucial role played by different membrane systems in regulating the intracellular Ca^{2+} and heart function, it has been considered that the inability of the failing heart to generate contractile force adequately is due to remodeling of both SL and SR membranes. Since Gwathmey et al (88) indicated that an abnormality of intracellular Ca^{2+}

handling in human cardiac contractile failure, several investigators have shown Ca^{2+} imbalance in the myocardium under pathological states. Defects in both SL Na^{+}-Ca^{2+} exchange as well as SR Ca^{2+}-pump ATPase and Ca^{2+}-uptake have been shown in heart failure due to myocardial infarction in rats (89, 90). It has been reported that the SR Ca^{2+}-uptake of myocardium obtained from patients with heart failure is diminished by 50% (91). The molecular mechanisms for changes in the SR membranes in cardiac hypertrophy and heart failure were explored by studying mRNA and protein levels for the SR ryanodine receptor, Ca^{2+}-pump ATPase, phospholamban and calsequestrin (92).

Several investigators have examined alterations in SR Ca^{2+} transport genes and proteins in pressure overloaded heart hypertrophy and failure (93-96). A depression in SERCA2 gene expression and SR function was observed together with a moderate increase in cardiac mass associated with major LV dilation, while no significant decrease of Ca^{2+}-pump ATPase mRNA level was detected when the increase in cardiac mass was about 20% (96). Although all studies showed a depression in SERCA2 mRNA expression in pressure overload hypertrophy model, no change of this gene was found in nonfailing heart or in mild cardiac hypertrophy (96-99). In parallel with the reduction of mRNA expression, SR Ca^{2+}-pump ATPase and phospholamban proteins were also found to be decreased in pressure overloaded heart (96, 97); no decrease in protein content of SR Ca^{2+}-pump ATPase was detected in mild hypertrophy (97). Calsequestrin mRNA level did not change in heart during development of cardiac hypertrophy and heart failure (98-102). In contrast, reduced calsequestrin has been reported in rabbit pulmonary artery constriction (95). The

experiments by Arai et al (99) have shown that the expression levels of mRNA for Ca^{2+} release channel, Ca^{2+}-pump ATPase and phospholamban were inversely correlated with the ANF mRNA level, suggesting that the expression of these mRNAs was decreased in severe heart failure. Schwinger et al (103) showed that the protein levels of SERCA2 and phospholamban were unchanged even though mRNA levels for SERCA2 and phospholamban in the failing heart were reduced in comparison to the nonfailing myocardium. These studies demonstrate that altered expression of SR genes is a major cause of altered Ca^{2+} handling seen in heart failure and cardiac hypertrophy, and the decrease in SR Ca^{2+}-pump ATPase gene expression may serve as a marker of SR remodeling in heart failure. Although altered mRNA expression of SR Ca^{2+}-pump ATPase and phospholamban has been reported in animal models of cardiomyopathy, only limited studies on the coronary occlusion model of heart failure are available in the literature. Recently Zarain-Herzberg et al (104) have shown a decrease in the SERCA2 gene expression in rats with heart failure due to coronary occlusion, but no changes in mRNA levels for ryanodine receptor, calsequestrin and phospholamban were detected. It should be pointed out that no changes in protein content of SR Ca^{2+}-pump ATPase, ryanodine receptor, phospholamban and calsequestrin have been reported in this animal model.

As indicated earlier, SL Na^+-K^+ ATPase isoforms are expressed in a tissue- and species dependent manner and their relative abundance change under various pathophysiological conditions. Cardiac right ventricular hypertrophy caused by experimental partial constriction of the pulmonary artery in cats reduced the density of Na^+-K^+ ATPase in

right ventricle without any effect in left ventricle (105). Heart failure induced by rapid ventricular pacing in dog was associated with a reduction of the α_3-isoform protein without any change in the α_1-isoform of Na^+-K^+ ATPase; this pattern was similar to that induced by norepinephrine infusion (106). In pressure overloaded rats produced by abdominal aortic constriction, α_2-isoform Na^+-K^+ ATPase was reduced in the early stages of hypertrophy whereas in severe hypertrophy α_3-isoform is increased (107). Shamraj et al (108) suggested there is a shift of α_1 to α_3 in the failing human heart. Ouabain (10^{-4}) sensitive K^+-uptake was decreased by 23.5% in hypertrophied myocytes compared to control (109). A depression in the Na^+-K^+ ATPase α_2- and α_3-isoforms was also observed in pig congestive cardiomyopathy induced by tachycardia (110). On the other hand, gene expression for the SL Na^+-Ca^{2+} exchanger was reported to increase in the failing human heart (111). Such changes in SL Na^+-K^+ ATPase and Na^+-Ca^{2+} exchanger gene expression reflect remodeling of the SL membrane during the development of heart failure.

Extensive studies on SR and SL membranes prepared from hypertrophied and failed animal and human hearts have suggested Ca^{2+}-handling abnormalities (2, 112-115). While some work regarding changes in SR gene expression in failing hearts from both experimental animals and humans (Tables 2 and 3) has appeared in the literature (92), relatively little is known about SL gene expression in the failing heart. Furthermore, alterations in SR cardiac gene expression are of selective nature and appear to depend upon the stage and type of heart failure (92, 116-120). Because of the activation of renin-angiotensin system in congestive heart failure, it is possible that angiotensin II may modify the gene expression for SL and SR

proteins and thus may result in remodeling of these membranes and Ca^{2+}-handling abnormalities in the failing heart. It is therefore appropriate to review the effects of renin-angiotensin system activation as well as its inhibition on heart function under some pathophysiological conditions.

4. Renin-angiotensin system and heart function

Although the renin-angiotensin system (RAS) was discovered in 1898 by Tigerstedt and Bergman (121), no attention was paid to this major cardiovascular control mechanism until 1934 when Goldblatt et al. (122) developed a reproducible model showing that the renal pressor substance is an enzyme. The term "angiotensin" was coined in 1958 for the active end product of the renin-angiotensin system whereas the importance of this vital neuroendocrine system was only recognized in hypertension and heart failure upon the availability of angiotensin converting enzyme (ACE) inhibitors in the late 1970s (123, 124). The classic view of the RAS is based on the premise that various components are derived from different organs and are in turn delivered to their site of action via the circulatory system. The primary components are: (a) angiotensinogen - a large globular protein that is secreted as the substrate for renin; (b) renin - an enzyme that catalyzes the proteolytic conversion of angiotensinogen to the decapeptide angiotensin I; (c) angiotensin converting enzyme - a dipeptidyl carboxypeptidase that converts angiotensin I to angiotensin II (Ang II) by cleavage of the two carboxyterminal amino acids; (d) Ang II - a highly active octapeptide

Table 2: Alterations of sarcoplasmic reticulum Ca^{2+} transport mRNA and protein levels in animal cardiac hypertrophy and heart failure

	mRNA level				Protein Level			
	SERCA2	PLB	RYR	CQS	SERCA2	PLB	RYR	CQS
Kiss et al. (95)	----	----	----	----	↓	↓	----	----
Matsui et al. (94)	↓	↓	↓	↓	----	----	----	----
Nagai et al. (93)	↓	↓	----	----	----	----	----	----
de la Bastie et al. (96)	↓	----	----	----	↓	----	----	----
Zarain-Herzberg et al. (104)	↓	↓	↓	----	----	----	----	----
Feldman et al. (98)	↓	----	----	----	----	----	----	----
Arai et al. (97) (mild cardiac hypertrophy)	↑	----	↑	----	----	----	----	----
Arai et al. (97)	↓	----	↓	----	----	----	----	----

SERCA2: sarcoplasmic reticulum Ca^{2+} ATPase; PLB: phospholamban; CQS: calsequestrin; RYR: ryanodine receptor; ↑: increase; ↓: decrease.

Table 3: Alterations of sarcoplasmic reticulum Ca^{2+} transport mRNA and protein levels in human heart failure

	mRNA level				Protein Level			
	SERCA2	PLB	RYR	CQS	SERCA2	PLB	RYR	CQS
Arai et al. (99)	↓	↓	↓	↔	----	----	----	----
Schwinger et al. (103)	↓	↓	----	----	↔	↔	----	----
Movsesian et al. (116)	----	----	----	----	↔	↔	----	↔
Meyer et al. (117)	----	----	----	----	↓	↓	↔	↔
Linck et al. (118)	↓	↓	----	----	----	----	----	----

SERCA2: sarcoplasmic reticulum Ca^{2+} ATPase; PLB: phospholamban; CQS: calsequestrin; RYR: ryanodine receptor; ↑: increase; ↓: decrease; ↔: no change.

and (e) Ang II receptors - specific receptors in the cell membrane upon which Ang II acts to produce physiological actions.

Renin, the rate-limiting enzyme of the cascade leading to Ang II formation, is an aspartyl protease with a molecular mass between 37,000 to 40,000. Its primary structure contains double domains; the amino- and carboxyl-termini contain areas of similar sequence (125). Renin is widely distributed and mRNA repression of renin can be found in kidney, adrenal, heart, ovary, testis, lung and adipose tissue (126); however, the main source of renin is the kidney (127). Human renin is cooled by a 12.5 kb DNA gene. On the other hand, angiotensinogen is an α_2-globulin with a molecular weight of 54,000 to 60,000. It is the only known substrate for renin and is the only known precursor for angiotensin peptides in vivo. There is only a single gene of 13 kb for angiotensinogen (128) and the majority of the circulating angiotensinogen is secreted from the liver. It is pointed out that ACE is a zinc metallopeptidase (129) that catalyzes the conversion of angiotensin I to Ang II as well as the breakdown of a broad range of substrates such as bradykinin (130). There exist two isoforms of ACE namely somatic ACE and germinal ACE. Both isoforms exhibit similar enzyme activities, but differ in molecular size and immunological properties (131). The ACE gene has been cloned in animals and humans, and it codes for a molecular weight ranging from 90 to 160 kD in different tissues (132, 133). This gene has been shown as an insertion/deletion polymorphism based on the presence of insertion (I) or deletion (D) in intron 16 of the ACE gene. This structure results in three genotypes: *DD* homozygous, *II* homozygous and *ID* heterozygous (134). The *DD* allele is associated with higher levels of

ACE in plasma (135) and is considered to increase the risk of cardiac disease (136-138). Ang II is considered to be an important factor for the regulation of vascular tone, blood flow and cardiac function. There is evidence to show that there are multiple biochemical pathways for the formation of Ang II (139, 140). These pathways may include the direct synthesis of Ang II from angiotensinogen (141) or another non-ACE enzyme such as chymase (142-144), which has been demonstrated to be present in the heart. The existence of non-ACE pathways suggests that long term therapy with ACE inhibitors may not lower the plasma and tissue Ang II levels appreciably (145) despite effective normalization of blood pressure and significantly suppressed ACE activity (146). The distribution of ACE and chymase in the heart differs; ACE is in the cardiac luminal surface whereas chymase is in endothelial cells and cardiac interstitium (147, 148).

By using specific non-peptide antagonists, two Ang II receptors have been identified as AT_1 and AT_2 (149, 150) and cDNAs encoding each type of Ang II receptors has been identified (151). The location of genes for AT_1 and AT_2 is different; the gene for AT_1 receptor is located on chromosome 3 whereas the AT_2 receptor gene is on the X chromosome (152). The Ang II receptor gene structure, distribution and regulation in different pathophysiological conditions has been fully reviewed (151, 153). Ang II receptors are up- and down-regulated by some biophysical mechanisms such as internalization and phosphorylation as well as disease conditions (154-156). The structure specificity of Ang II receptor is high; the affinity for binding to Ang II is similar to Ang II circulating concentration (10^{-10} M). The AT_1 receptor is a seven-transmembrane receptor with two

subtype receptors, AT_{1A} and AT_{1B}; these subtypes have similar polypeptides, containing about 360 amino acids, but have different tissue distribution (139, 157). This class of Ang II receptors is associated with G_q protein (158), and is responsible for almost all the physiological actions of Ang II and selective antagonists. The AT_1 receptor can initiate either a rapid or a slow signal transduction event. In the rapid signal transduction event, the phosphoinositide message system is involved (159). Ang II through the type-1 receptors activates Ca^{2+} channels through G_q proteins to allow more Ca^{2+} into the cells. In turn, phospholipase C is activated to generate inositol bisphosphate which activates protein kinase C (PKC) and finally regulates cell function (160). The slow signal transduction event involves the phosphorylation of tyrosine and activation of mitogen-activated protein kinase (MAP kinase) which stimulates cell growth and causes hypertrophy (161-163). All the effects induced by Ang II can be blocked by losartan indicating that functional activity is mediated mainly by the AT_1 receptor. On the other hand, the AT_2 receptor is quite different from the AT_1 receptor; the AT_2 receptor is blocked by compound PD123319, a selective AT_2 receptor antagonist (153). The function of the AT_2 receptor is not yet clear. Recently, growing evidence has shown that AT_2 receptor is also involved in functional activity. PD123177, a related compound, has been shown to delay and attenuate the Ca^{2+} spike induced by Ang II in cultured bovine adrenal medullary cells. By using PC12W cells, which express high levels of AT_2, but not AT_1 receptor, Yamada et al. (164) recently reported that the AT_2 receptor involves dephosphorylation of MAP kinase and results in apoptosis. This AT_2-mediated MAP kinase dephosphorylation and apoptosis can be blocked by vanadate and

an antisense oligonucleotide to MAP kinase. Another study indicates that the AT_2 receptor is regulated by PKC-calcium pathway; the increase of Ang II receptor gene expression was inhibited with a PKC inhibitor (165).

A. Cardiac renin-angiotensin system

Multiple lines of biochemical and molecular evidence support the existence of a local RAS (166-172). The most convincing evidence for a cardiac RAS is the expression of renin, angiotensinogen and ACE genes in cardiac tissues (173-176). The renin and angiotensinogen mRNA has been shown to exist in all four chambers of the heart with a different distribution depending on species and pathophysiological conditions (177). In contrast, von Lutterotti et al. (178) indicated that renin is not synthesized by cardiac tissue and that the local RAS accumulates renin from the bloodstream. Angiotensin I and II can be detected in the isolated rat heart when renin is added to a perfusion buffer; this means that angiotensinogen and ACE, but not renin, exist in isolated heart tissues (179). Experiments have indicated that ACE is not uniformly distributed in the heart. By using ^{125}I-351A as a radioligand, Yamada et al. (180) demonstrated that in rat heart the highest density of ACE is in valve leaflets and the lowest is in endocardium. Upon combining in vitro autoradiography with an examination of tissue morphology, Sun et al. (181, 182) showed that low density ACE was found throughout the ventricular myocardium, whereas high density of ACE exists at the site of high collagen turnover, including heart valve leaflets. The distribution of ACE in the heart indicates that some Ang II is possibly generated in the heart. In fact, cardiac Ang II production has been demonstrated (142, 183). Receptors which are related to the function

of Ang II have been characterized in the cardiovascular system (154, 184). It is thus likely that local Ang II plays an important role in cardiovascular homeostasis in autocrine and paracrine fashions and may be involved in cardiac remodeling.

B. Effect of Ang II on cardiac function

Ang II influences cardiac function by affecting cardiac contraction, myocytes, cardiac matrix growth and cardiac metabolism. These actions are initiated by the binding of Ang II to a plasma membrane receptor that stimulates phospholipase C (PLC) to produce hydroxyl phosphatidylinositol 4,5-bisphosphate and thus forming diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3). The subsequent rise in intracellular Ca^{2+} that results from IP_3-mediated release of Ca^{2+} from intracellular stores, together with DAG, activate PKC. In cultured rat ventricular myocytes, Ang II not only activates the phosphoinositide pathway, but also activates the phospholipase D and A_2 pathways (161, 185). Ang II has long been recognized to influence cardiac contractility (186-188). A positive inotropic effect of Ang II was reported on isolated neonatal rat cardiomyocytes (189, 190), pithed rabbit preparations (191) as well as perfused rabbit and cat hearts (191, 192). Under in vivo conditions in rabbits, Zhang et al. (193) have shown that Ang II elicited a dose dependent increase in blood pressure, left ventricular pressure, rate of contraction (+dP/dt) and rate of relaxation (-dP/dt) as well as heart rate. The increase in both +dP/dt and -dP/dt by Ang II was confirmed in isolated rabbit hearts (194) and rat myocytes (195).

It may be noted that Ang II was found to increase interleukin-1 induced nitric oxide synthesis; this effect was blocked by a protein kinase C inhibitor, calphostin (196). Unlike

β-blocker or diuretics, the reduced levels of cholesterol and lipoprotein by an ACE inhibitor were increased by Ang II (197, 198). Ang II interacts with the sympathetic nervous system through presynaptic transmitter release causing an improvement of the baroreceptor reflex function (199, 200). Interactions between the renin-angiotensin system and parasympathetic nervous system in heart failure are also observed. Heart failure patients show a reduction in vagal tone (201), and baroreflex sensitivity, associated with an increased plasma renin activity (202, 203). Thus it appears that the action of Ang II in cardiovascular system is both direct and indirect.

C. Effect of Ang II on Ca^{2+} mobilization

Ang II has been reported to be involved in Ca^{2+} mobilization in ventricular myocytes through the activation of slow calcium channels in the sarcolemmal membrane (190). Allen et al. (189) have observed that Ang II can stimulate contractile frequency and calcium sensitive calcium current. Arnaudeau et al. (204) indicated that angiotensin AT_1 receptor stimulates Ca^{2+} sparks through activation of L-type Ca^{2+} channels without involving IP_3-induced Ca^{2+} release; this stimulatory effect was blocked by a PKC inhibitor but not by propranolol (205). Ang II induced the cytosolic free calcium increase in chick myocytes in a dose dependent manner (206). Unpublished data from our laboratory have revealed that Ang II can increase intracellular Ca^{2+} in isolated adult rat myocytes in a dose dependent manner; this effect was abolished by both Ang II receptor antagonist losartan and PD123319. Although Ang II can be seen to cause an increase in free Ca^{2+} in the myocytes, the results are controversial. Ang II (10^{-8} M) induced a significant increase of fractional shortening which

was not associated with an increase of calcium transient or any effect on L-type calcium inward current (207). In isolated rabbit myocytes, Ang II stimulated the rates of contraction and relaxation but failed to show any increase in intracellular Ca^{2+}. On the other hand, in neonatal rat heart myocyte cultures, the frequency of contraction and Ca^{2+} current were increased when a protein kinase C (PKC) activator, phorbol ester 12-0-tetradecanoylphorbol-13-acetate (TPA), was added to the buffer containing Ang II; this effect was not seen with 4-α-phorbol-12,13-didecanoate (α-PDD), which does not activate PKC (208).

D. Ang II-induced cardiac hypertrophy and heart failure

Cardiac growth is affected by mechanical load and neurohumoral substances, such as Ang II, which acts as an endogenous growth factor. Ang II stimulates cardiac growth that is involved with myocyte hypertrophy as well as growth of non-myocytes such as collagen and fibronectin. During hypertrophy, Ang II has been shown to stimulate protein synthesis, DNA synthesis, secretion of growth factors and formation of cardiac matrix (209-214). Ang II, at 10 μM concentration, increased collagen and fibronectin synthesis and their mRNA expression in cultured rat vascular smooth muscle cells (214). In neonatal rat cardiac fibroblasts, 24 hour exposure to 1 μM Ang II increased the rates of phenylalanine, thymidine and uridine by 58%, 103% and 118%, respectively (215). Intracellular signaling pathways of Ang II may include: (a) phosphatidylinositol message pathway, (b) tyrosine kinase pathway via Ras/Raf pathway to activate the protein kinase and (c) cascade to activate MAP kinase. These events in sequence may stimulate the growth factor dependent c-*fos*, c-*jun* and *Egr*-1, increase in transcription. Although these intracellular mechanisms are stimulated by

Ang II due to its interaction with angiotensin receptors present in the cell membrane, the contribution of each receptor type (AT_1 and AT_2) is far from understood. It should be mentioned that cardiac hypertrophy is an adaptive response to an increased load on the myocytes which allows the heart to perform increased work in the presence of normal systolic fiber shortening (216). On the other hand, heart failure is a complex syndrome in which a number of subcellular biochemical alterations have been identified (217, 218). However, there is a real challenge for understanding events associated during the transition of cardiac hypertrophy to heart failure.

By using chick heart cells, Baker and Aceto (210) found that Ang II significantly stimulated protein synthesis through the participation of AT_1 receptors. Under the same experimental conditions it was shown that Ang II-induced protein synthesis was time and dose dependent (210). Likewise, Greenen et al. (211) demonstrated that Ang II increased cardiac protein synthesis in adult rat heart. The work from Schunkert et al. (219) not only confirmed that Ang II stimulated protein synthesis in adult rat hearts directly but also explained that this effect of Ang II on protein synthesis was mediated through AT_1 receptors and the activation of PKC. The Ang II-induced ventricular hypertrophy was not a consequence of high blood pressure because lowering the blood pressure or vasodilator therapy did not regress the cardiac hypertrophy (220). The intracardiac angiotensin I to II conversion was fourfold higher in hypertrophied rats due to an increase of the ACE activity; these changes were reversed by an ACE inhibitor, suggesting that ACE is a key enzyme involved in cardiac hypertrophy (219). It should be pointed out that administration of an

ACE inhibitor not only prevented cardiac hypertrophy but also caused a regression of the previously developed left ventricular hypertrophy. The recently established new transgenic (TGR9nRen2) rat was an ideal model to demonstrate the direct effect of the renin-angiotensin system on cardiac hypertrophy (221). This transgenic rat has been shown to develop hypertension, which can be normalized by an AT_1 receptor antagonist (222, 223).

The formation of cardiac extracellular matrix has been demonstrated to be increased by the renin-angiotensin system (224, 225) and appears to play an important role in the transition from hypertrophy to heart failure (226). A marked ACE binding in rats after coronary ligation was associated with fibrillar collagen formation in the infarcted and remote areas (182). In cultured cardiac fibroblasts, Ang II induced an early growth response (*Egr*-I) gene as well as increased mRNA levels for c-*fos*, fibronectin and laminin 2- to 4-fold (227). Not only collagen is a major component of the extracellular matrix but the accumulation of fibrillar collagen in the cardiac interstitium is also the major morphological feature of ventricular hypertrophy (228). The increase in collagen I and III contents in the myocardium was attenuated in the presence of AT_1 and AT_2 receptor antagonist (229). Infusion of Ang II was found to stimulate fibronectin gene expression accompanied with an increase in collagen I and IV gene expression in rat hearts (230, 231). Compared with other growth factors, Ang II showed a strong effect on the expression of early oncogenes, *Egr*-1 and extracellular matrix genes such as fibronectin and laminin (227). On the other hand, some investigators did not observe any change in collagen gene expression by Ang II (227). The pathway for Ang II mediated non-myocyte hypertrophy has been reviewed recently by Dostal

et al. (232).

E. Mechanisms of cardiac remodeling by Ang II

By stimulating the formation of extracellular matrix, Ang II is considered to alter the size and shape of cardiomyocytes and thus results in remodeling of the heart. In cultured rat cardiac cells, the c-*fos* mRNA expression was significantly induced by Ang II (232). The pressure overload induced c-*fos* expression was also found to be Ang II dependent. In stretched adult failing cardiomyocytes, c-*fos* mRNA was increased 3- to 4-fold after Ang II treatment; this increase and *c-fos* expression were blocked by AT_1 receptor antagonist (161). Late hypertrophy response, skeletal α-actin, atria natriuretic factor and protein synthesis, were suppressed by Ang II receptor antagonist (233). In myocardial infarction induced hypertrophy, c-*myc*, *c-jun* and Ang II receptor mRNA were increased significantly (234). In heart failure due to coronary occlusion, Ang II receptors, *c-myc* and *c-jun* were also increased in myocytes (235). Ang II has been shown to stimulate growth factor in adult cardiac fibroblasts (236). Ang II also caused a marked increase of insulin-like growth factor-I receptor gene expression and gene transcription in rat aortic smooth muscle cells (237). In rat cardiac hypertrophy induced by abdominal coarctation, an increase in AT_1 receptor mRNA was associated with two-fold increase of the transforming growth factor-β_1 mRNA; this increase can be blocked by an Ang II receptor antagonist, DuP 753 (238). Ang II stimulated MAP kinase by PKC dependent (239) or independent (240) pathways with an increase of intracellular Ca^{2+} and thus stimulating myocyte growth (241).

An increase in the activity and expression of the local RAS gene was evident in

cardiac hypertrophy and heart failure; this increase was associated with an increase in plasma renin activity by 45%, total RNA by 68% in rats with heart failure (242). The increased ACE mRNA level was accompanied by a decrease of AT_1 receptor mRNA to 46% without any change in AT_2 receptor mRNA in myocardium of decompensated rats (242). The cardiac renin, angiotensinogen, ACE, and AT_1 and AT_2 receptors were expressed in volume overloaded rat heart in which increases in renin, angiotensinogen as well as ACE mRNA, unlike mRNA for Ang II receptors, were evident (243). The level of ACE mRNA increased in the ventricles during cardiac hypertrophy by aortic banding and in the model of low output cardiac failure by coronary ligation (244, 245). In the hypertrophied rat heart, ACE mRNA was 2-fold more than that in the normal ventricle. Infusion of angiotensin I into the hypertrophied heart for 15 min caused intracardiac conversion of Ang I to Ang II; this increase was 4-fold compared to the sham control (219). The increase of Ang II receptor was not only evident at the site of myocardial infarction but also in fibrous tissues involved in myocardial infarction and pericardial fibrosis (246). In hypertrophied heart due to myocardial infarction, gene expression and protein content of renin, angiotensinogen, ACE as well as the angiotensin receptors increased significantly (247). On the other hand, a decrease in Ang II receptor mRNA was seen in patients with heart failure and this reduction was attenuated by losartan and PD123319 (248). Both AT_1 and AT_2 receptors were found to increase in injured and non-infarcted tissues, but only AT_1 antagonist attenuated this receptor change in rat after coronary artery ligation (249). Ang II receptors were down-regulated in pressure overload myocardial hypertrophy and heart failure in rats but up-

regulated in post infarcted cardiac hypertrophy and heart failure (250-253). Reduction of both Ang II receptors, with loss of AT_1 receptor mRNA, are noticeable in patients with end stage heart failure (254). In severe heart failure, plasma renin and plasma Ang II concentrations increased 3-fold. In parallel, renal renin and angiotensinogen mRNA expression also increased. These observations suggest that the renin-angiotensin system is activated in heart failure but the changes in its different components seem to depend on the type and stage of the disease (255).

From the foregoing discussion, it is clear that ACE genotype has a close relationship with cardiac hypertrophy and heart failure, and has also been implicated in cardiac dilatation and myocardial infarction (138, 256, 257). Since Cambine et al. (137) first reported a deletion polymorphism in the ACE gene (*DD*) which was associated with an increased risk of myocardial infarction, the relationship between ACE genotype and cardiac hypertrophy or heart failure has been studied more extensively (258-260). This relationship is demonstrated by: (a) higher occurrence of cardiac hypertrophy and heart failure in patients with a *DD* genotype; (b) more *DD* ACE genotype gene in hypertrophy or heart failure patients; (c) influence of the *DD* genotype on the survival following heart failure. Schunkert et al. (261) suggested that the *DD* genotype may act as a marker associated with an elevated risk of left ventricular hypertrophy in men, since men have a stronger association of the *DD* genotype than women. In contrast, no relationship of ACE genotype with cardiac hypertrophy was reported (262).

F. Effects of ACE inhibitors on cardiac hypertrophy and heart failure

Since the synthesis of first oral ACE inhibitor, captopril, in 1977 (123, 124, 263), several other ACE inhibitors have been synthesized and their effects on hypertension and heart failure have been fully investigated (264, 265). Although it is generally accepted that ACE inhibitors have a beneficial effect in heart failure, the mechanism of such a protective effect is still far from being fully understood (266, 267). The following discussion is devoted to analysis of the actions of some ACE inhibitors in different types of hypertrophied and failing hearts:

(i) Effect of ACE inhibitors on heart failure induced by myocardial infarction

Left ventricular infarction in rat has been used as an ideal model of cardiac hypertrophy and heart failure. The RAS is known to be activated during postinfarction and is thought to play an important role during the remodeling period. Therefore, this model has also been used to demonstrate the benefits of ACE inhibitors. Myocardial infarction has been characterized as a combination of pressure and volume overload in which the myocardium faces an excessive workload (268). Marked changes in ventricular hemodynamics, volume, and mass are related to infarct size. Cardiac function is lowered as characterized by lower output, reduced ejection fraction, elevated end-diastolic pressure, ventricular dilatation and ventricular hypertrophy which finally leads to heart failure.

Due to the fact that infarct size is an important factor influencing the process of the postinfarction and the occurrence of heart failure, attention has been paid to the fact that ACE inhibitors may reduce infarct size. Although several studies have examined the effect

of ACE inhibitors on infarct size, the results are controversial. Reduction of the infarct size by ACE inhibitors has been reported in dog (269-271), rat (272, 273) and cat (274). When the ACE inhibitor is administered 15 min to 6 hours after the coronary occlusion, infarct size reduction was evident in dogs and it was suggested that ACE inhibitors can reduce infarct size by increasing collateral flow to the areas of infarction as well as areas at risk (275). Treatment with captopril for 3 weeks starting 3 weeks after coronary artery occlusion reduced the infarct size only by 9% in rats (272). On the other hand, various studies failed to observe any change in infarct size upon ACE inhibitor therapy. In a dog model of coronary artery occlusion, captopril improved cardiac output significantly, but did not decrease the infarct size (276). In addition, ACE inhibitors failed to reduce infarct size in conscious dogs (277, 278). Both reduction and no change in the infarct size have been reported upon occluding coronary artery in rats (279, 280). Such a discrepancy in results seems to be due to the time of administration and dose of ACE inhibitor.

Improved systolic and diastolic function in both experimental animals and patients with heart failure has been well documented by the use of various ACE inhibitors, such as captopril (273, 281), enalapril (282), trandolapril (283), idrapril (280) and ramipril (284). Pfeffer et al. (285) were first to report that captopril significantly prevented the ventricular dysfunction. Captopril, given to infarcted rats for 3 weeks, showed a shortening of peak time tension, an increase in $\pm dP/dt$ and developed tension, and a particular reduction of myocardial stiffness (272). In general, ACE inhibitors improved ventricular hemodynamics, attenuated ventricular dilatation and reduced wall stress and stiffness. ACE inhibitors also

increased the baseline and maximum stroke volume index, cardiac output, and coronary circulation. Some investigators have failed to show the protective effect or even partial protective effects of ACE inhibitors. Although ACE inhibitors significantly improved cardiac function in heart failure, their effects on cardiac systolic and diastolic function are different. A recent study has demonstrated that long term ACE inhibitor treatment improved diastolic function more than systolic function. These data indicate that the diastolic filling abnormalities are almost completely normalized but diastolic dimensions and posterior thickening are left unchanged after a long term treatment with captopril (286).

Compared to other drugs such as vasodilators, β-adrenergic blockers and Ca^{2+} antagonists, ACE inhibitors demonstrate the greatest advantage in delaying the development of heart failure and increasing the survival rate. The effect of chronic ACE inhibition on long-term survival after myocardial infarction was first demonstrated in rats with myocardial infarction (273). Subsequently ACE inhibitors were used in clinical trials showing their beneficial effect on mortality and morbidity in heart failure (287-289). Some of the studies, however, have shown a negative action of ACE inhibitors (290). In a one year survival study, an ACE inhibitor, trandolapril, showed improved survival rate in myocardial infarcted rats only during the initial 6 month period (291). Our experiments with a new long acting ACE inhibitor, imidapril, have shown that ACE inhibitors may produce beneficial effects irrespective of the time of treatment following coronary occlusion. Both early (1 hour after coronary artery occlusion) or late (3 weeks after coronary artery occlusion) treatments of rats reduced the mortality compared to that in the respective untreated infarcted group. The

mechanism by which ACE inhibitors improve survival following early or late myocardial infarction may however be different (unpublished data).

Remodeling of the heart subsequent to myocardial infarction is characterized by progressive left ventricular dilatation and enlargement of the chamber size. At the cellular level, remodeling of the heart may include changes in membranes, contractile proteins and cardiac matrix. ACE inhibitors appear to prevent the occurrence of cardiac remodeling in heart failure which is currently well reviewed (292-294). Early treatment with ACE inhibitor was found to prevent the progression of left ventricular remodeling in dogs with left ventricular dysfunction caused by sequential intracoronary embolizations with polystyrene latex microspheres (295). In addition, the beneficial effects of ACE inhibitors on cardiac remodeling were also seen in noninfarcted regions of the myocardium (296) and large arteries (297). Ramipril limited the decline in function in noninfarcted regions and prevented the percent circumferential shortening in the subendocardium when given to sheep for 8 weeks starting two days after coronary occlusion (296). Captopril significantly reduced collagen levels and reduced the artery media thickness which was accompanied by improved hemodynamic functions in the coronary occluded rats (297). Although ACE inhibitors are considered to prevent cardiac remodeling by affecting the size and shape of hypertrophied myocytes by decreasing the formation of cardiac matrix, ramipril did not normalize the elevated collagen content in rats with myocardial infarction (298). The multiple factors that may contribute to the action of ACE inhibitors caused by remodeling of the heart are as follows: decrease of hemodynamic load, increase in bradykinin levels, regression of myocyte

hypertrophy and a decrease in collagen accumulation (294).

In spite of the evidence that ACE inhibitors improve cardiac function, delay the occurrence of heart failure and prolong survival, the time of administration and doses of these agents are still in discussion (299, 300). Treatment of rats with idrapril before coronary occlusion showed a marked improvement in left ventricular function and prevention of cardiac remodeling (280). A recent report stressed the importance of early ACE inhibitor treatment (300). Early administration of lisinopril to patients with acute myocardial infarction showed a long term benefit of survival rate (291). On the other hand, an earlier study demonstrated that late, but not immediate, treatment with captopril improved cardiac function following heart failure subsequent to coronary occlusion (301). The doses used for treatment also affected the beneficial influence of ACE inhibitors (302). In rats with heart failure after myocardial infarction, one year survival rate improved with high doses, but not with low doses, of lisinopril (303). In view of the differences in the molecular structure of the two binding sites of ACE (304), it is possible that different ACE inhibitors may interact with ACE at one or both sites and this may explain the differences in the time and dose dependent effects of these agents.

(ii) Effects of ACE inhibitors on cardiac hypertrophy and heart failure due to volume overload

Heart failure due to volume overload is different from other types of heart failure as it is characterized by an eccentric pattern of hypertrophy and dilation of the ventricular cavities. The effects of ACE inhibitors on heart failure due to volume overload have not

been studied extensively; however, a recent review has discussed the status of volume overload hypertrophy and heart failure (305). There is a characteristic elevation of plasma atrial natriuretic factor (ANF) due to increased release and synthesis of ANF in volume-induced heart failure (306). Winkins et al. (307) suggested that ANF could be used as a good indicator of cardiac volume overload in aortocaval fistula because plasma ANF is correlated to the degree of cardiac hypertrophy and urinary excretion of cGMP. Arnal et al. (308) observed that perindopril exerted a beneficial effect on cardiac hypertrophy and suggested that ACE inhibitors may regress cardiac hypertrophy mainly via their effect on the pressure load rather than on the volume load. By combining the pressure and volume overloads, Takeda et al. (309) showed that captopril significantly increased the tension (dT/dt_{max}) and attenuated the myosin isozyme shift. Furthermore, the fact that ACE inhibitors significantly attenuated the ventricular ACE mRNA expression as well as the mRNA of AT_{1A} and AT_{1B}, supports the idea that RAS is involved in volume overload heart failure (245). Treatment of rats with enalapril for 7 weeks significantly reduced the increased LVEDP due to volume overload (310, 311). Similar results were also seen upon captopril treatment for a period of 3 weeks (312). The improved hemodynamics associated with a regression of cardiac hypertrophy indicated that the renin-angiotensin system may exert some direct effects on volume overload cardiac hypertrophy (313). Since the changes in extracellular matrix are invariably observed in hypertrophied hearts, the effects of ACE inhibitors on collagen and elastin have been investigated in volume overload cardiac hypertrophy. In contrast to other forms of cardiac hypertrophy, collagen was reduced in volume overloaded hypertrophied left

ventricle and this reduction was attenuated by enalapril treatment. Enalapril also blocked the initial increase of elastin in the same model (313). Although ACE inhibitors reduced the increase in ANF levels following heart failure due to coronary occlusion, no such reduction was seen in the volume overload heart failure in spite of the beneficial effects on hemodynamics (312). Differential effects of ACE inhibitors on circulating versus cardiac Ang II appear to explain the differences in the beneficial effects of various agents on cardiac hypertrophy and hemodynamic changes due to volume overload (314, 315).

(iii) Effect of ACE inhibitors on cardiac hypertrophy and heart failure due to pressure overload

Cardiac hypertrophy due to pressure-overload (a concentric hypertrophy) is characterized by a concentric increase in wall thickness with no increase in chamber radius or volume. The left ventricle has been shown to increase by about 50% within 6-12 weeks of aortic banding in rats (316). Significant prevention of cardiac hypertrophy by the use of different ACE inhibitors has been demonstrated in the rat aortic stenosis model (317-319). ACE inhibitors not only produced a regression of cardiac hypertrophy but also prolonged the survival of rats with aortic stenosis (320). Hemodynamic measurements showed that although the left ventricular systolic pressure was still high after treatment of pressure-overloaded rats with fosinopril, the left ventricular diastolic pressure was markedly reduced (321). Assessment of the left ventricular geometry and function in rats with aorta banding revealed that fosinopril prevented the increase in left ventricular cavity size, increased the left ventricular wall stress and attenuated the systolic and diastolic functions due to pressure

overload (321). Both ramipril and enalapril were beneficial in regressing cardiac hypertrophy due to constriction of the abdominal aorta in rats regardless of whether the administration was immediate or 3 weeks after the operation. Ang II receptor antagonists also reduced cardiac hypertrophy but to a much lesser degree in comparison to the reduction following surgical removal of aorta banding (322).

(iv) ACE inhibitors on heart failure due to pacing or dilated cardiomyopathy

The direct benefit of ACE inhibitor on cardiac tissue was determined by using different models of cardiomyopathy. In pacing-induced cardiomyopathy, fosinopril not only improved cardiac function, but also improved the myocytes velocity of shortening after β-adrenergic receptor stimulation as a consequence of an increase in β-adrenergic receptor density (323). Captopril treatment maintained normal cardiac output and pulmonary capillary wedge pressure after heart failure caused by rapid right ventricular pacing (324). The beneficial effect of ACE inhibitors on cardiomyopathy may be through the elevation of circulating angiotensin I (325). ACE inhibitors decreased cardiac collagen accumulation differently in various strains of cardiomyopathic hamsters. Masutomo et al. (326) demonstrated that enalapril significantly decreased collagen concentration, the ratio of collagen 1 to 3 as well as collagen 3 mRNA expression in BIO14.6 strain, but not BIO53,58 strains of cardiomyopathic Syrian hamsters.

G. Possible mechanisms of the beneficial effects of ACE inhibitors

(i) Free radical scavenging properties

Some experiments have provided evidence regarding the radical scavenger properties

of ACE inhibitors containing sulfhydryl group. In an early study, Chopra et al. (327) suggested that captopril may act as a powerful free radical scavenger. In their study, free radicals were generated by photo oxidation of dianisidine sensitized by riboflavin, and captopril was shown to possess a scavenging ability in a dose dependent manner. Captopril was also demonstrated to exert a powerful effect in scavenging superoxide anion radicals, hydroxyl radicals and hypochloride radicals (328). The limitation of labeling ACE inhibitors as free radical scavengers lies in the fact that not all ACE inhibitors contain the sulfhydryl group and yet these agents have a similar protective effect on cardiac functions.

(ii) Cellular mechanisms

Change in intracellular calcium handling is shown to occur in heart failure, and the beneficial effect of ACE inhibitors for improving cardiac function is possibly associated with improvements of the sarcoplasmic reticulum Ca^{2+} ATPase gene expression in renal hypertensive rats (329). ACE inhibitors have also been shown to improve the response to Ca^{2+} stimulation in hypertrophied myocytes which may be important for preventing the transition from compensated hypertrophy to heart failure (330). In addition, we have shown that ACE inhibitors improved β-adrenergic receptor transduction by increasing the decreased β_1-adrenergic receptor density, the decreased adenylyl cyclase activity, and attenuating G-protein changes in the failing hearts (unpublished observations). Sanshi and Takeo (331) have also reported that long term trandolapril treatment significantly attenuated the cardiac β-adrenoceptor response in rat with heart failure following coronary occlusion.

(iii) Effect on bradykinin

Besides the inhibition of renin angiotensin system, the elevation of bradykinin levels might be responsible for some beneficial effects of ACE inhibitors. This view is supported upon administrating a bradykinin antagonist which abolished the protective role of ACE inhibitors in ischemic heart (332) and the regression of cardiac hypertrophy by ramipril in hypertensive rats (333). The effect of ACE inhibitors involves bradykinin-mediated actions which include increasing the coronary blood flow, improving the left ventricular pressure, decreasing the arterial ventilation and antiproliferating properties (334, 335). It should be noted that bradykinin is a vasodilator which acts by increasing the release of endothelium-derived factors such as nitric oxide and prostacyclin. Bradykinin may also improve the status of high energy phosphates in ischemic myocardium (335, 336). Although the protective effect on cardiac function and regression of cardiac hypertrophy by ACE inhibitors can be considered as a part of the function of bradykinin, this positive effect, however, is not related to the equally effective Ang II receptor antagonists.

(iv) Effect on myosin heavy chain

There exists a positive relationship between myosin heavy chain and cardiac muscle contractility. Reduced myosin heavy chain content and the isoform shift in heart failure can be attenuated by ACE inhibitor treatment. In our laboratory, rats with heart failure induced by coronary occlusion showed lower myosin heavy chain content and shifted altered myosin isoform gene expression. By using the ACE inhibitor, imidapril, for 4 weeks, a significant improvement of myosin heavy chain content and normalization of the myosin isoform shift

were observed (unpublished data). Lambert et al. (337) also showed that perindopril significantly limited the shift of isomyosin in the cardiomyopathic Syrian hamster. Michel et al. (338) showed that the treatment of myocardial infarcted rats with an ACE inhibitor over a 2 month period significantly attenuated the isoform shift.

(v) Effect on neuroactivity

Diminution of parasympathetic tone associated with enhanced arrhythmogenesis and sudden cardiac death is a feature of congestive heart failure (201). ACE inhibitors have been shown to exert vagomimetic action in congestive heart failure (339). ACE inhibitors significantly increased the baroreflex sensitivity in patients with idiopathic dilated cardiomyopathy and coronary artery disease (339). Captopril significantly attenuated the depressed baroreflex sensitivity in patients with acute myocardial infarction (340). Although ACE inhibitors were known to affect sympathetic activity, no action of ACE inhibitors on healthy subjects was observed (341).

(vi) Effect on energy metabolism

Lactate dehydrogenase (LDH) and its isoenzymes are closely related to aerobic and anaerobic metabolism. Shifts of lactate dehydrogenase isoenzymes are dependent on the state of the oxygen supply and may serve as a marker for the energy state of the myocyte; LDH1 acts as a marker for the aerobic state whereas LDH5 as a marker for the anaerobic state (197, 342). In cardiac hypertrophy and heart failure, reduction of available energy is evidenced by changing the isoforms of LDH, or decreasing the ADP/ATP ratio; this shift in the LDH isoenzyme and alteration of the ADP/ATP ratio can be interpreted to reflect the

beneficial effects of the ACE inhibitor therapy. Treatment with enalapril for 6 months shifted LDH towards LDH1, and the ADP/ATP carrier concentration increased to normal levels; accompanying these changes, a significant effect on the hemodynamic index was also observed. Although this study indicated that ACE inhibitors had a protectice effect on metabolism, it is not clear whether the change in energy metabolism is a cause or a consequence of the hemodynamic alterations (339). Enalapril significantly increased LDH1 concentration, preserved myocardial creatine kinase and improved the survival of rats with heart failure (343). The study by Zhu et al. (336) indicated that ACE inhibitors improve metabolism via a mechanism that involves bradykinin rather than Ang II inhibition. On the other hand, ACE inhibitors such as captopril, enalapril and ramipril significantly improved the myocardial oxygen consumption in dogs by increasing nitric oxide accumulation (344).

H. Effects of Ang II receptor antagonist on cardiac hypertrophy and heart failure

In view of the indirect evidence that the RAS is at least partially responsible for the progression of heart failure, this system is considered to influence the prognosis in heart failure. The discovery of the Ang II receptor antagonists has provided an adequate tool for studying the role of Ang II receptors and the renin-angiotensin system in heart function. By using Ang II receptor antagonists one can avoid to some extent the side effects caused by ACE inhibitors. Furthermore, by blocking Ang II with nonpeptides that lack agonist activity it is now possible to confirm that the efficacy of ACE inhibitors is due to a decrease in the Ang II level instead of an increase in the bradykinin level. The earliest Ang II antagonist was reported to block Ang II receptors and reduce blood pressure but was found to exhibit some

Ang II agonist activity (345). The first non-peptide AT_1 receptor antagonist, losartan, was discovered to possess agonist activity (346-348). Losartan was found to affect the Ang II receptors by interacting with amino acid in the transmembrane domains of AT_1 receptors, occupying space among seven helixes and thus preventing the binding of Ang II (349).

Losartan is a novel, orally active, non-peptide Ang II receptor antagonist that blocks the Ang II receptors. In an early study using rats with heart failure following coronary occlusion, Raya et al. (350) found the beneficial effects of both Ang II blocker and ACE inhibitor with respect to changes in LVEDP, left ventricular end diastolic volume and the venous compliance. Smits et al. (351) later showed that both early and late treatments with losartan following myocardial infarction were beneficial in modifying the changes in the central venous pressure as well as in inhibiting the collagen deposition and regressing the cardiac hypertrophy. However, losartan failed to show any beneficial effect with respect to changes in cardiac output and inhibiting DNA synthesis in the failing ventricle. Nonetheless, losartan has been used in heart failure patients and clinical data confirm the beneficial effect for lowering the systemic vascular resistance and increasing the cardiac output (352, 353). Furthermore, short-term administration of losartan has been shown to significantly improve impaired cardiac function, reduce systemic vascular resistance as well as pulmonary capillary wedge pressure, and increase cardiac index (353). In addition to having beneficial effects in heart failure due to myocardial infarction, Ang II receptor antagonists have been shown to exert beneficial actions in volume overload-induced (310), pressure overload-induced (322) and pacing-induced heart failure (354). The Ang II receptor antagonist TCV-116 at

a dose of 10 mg/kg/day significantly reduced the increased left ventricular weight and left ventricular thickness due to pacing of the heart (355). As well, this treatment attenuated the shift of the beta myosin heavy chain isoforms and inhibited cardiac hypertrophy by inhibiting the [^{3}H]phenylalanine incorporation, MAP kinase activity and the c-*fos* expression induced by stretch of cardiomyocytes. Losartan has also been shown to attenuate the altered response of myocytes to Ang II in heart failure due to pacing (356). Since cardiac remodeling after myocardial infarction has a close relationship with the expression of the phenotype genes, the modulation of cardiac phenotype gene expression by TCV-116 revealed beneficial effects on remodeling of cardiac tissue (356). Attenuation of ventricular dilatation after myocardial infarction by TCV-116 (357) indicates that Ang II antagonists are capable of delaying the development of heart failure after myocardial infarction.

Although there are many similar effects of ACE inhibitors and Ang II receptor blockers, some differences exist between the two classes of drugs. ACE inhibitors prevent the inactivation of bradykinin. ACE inhibitors do not completely inhibit the production of Ang II pathways whereas Ang II receptor antagonists directly block the action of Ang II by interfering with its receptor binding (358). Losartan has tissue specific effects on endogenous levels of angiotensin and bradykinin, but the increase of bradykinin does not contribute to the action of losartan (359). Losartan (10 mg/kg) was found to increase plasma renin and cardiac Ang II, decrease plasma angiotensinogen and increase plasma ACE, but does not increase the tissue ACE levels (360). It should be pointed out that there exist some discrepancies concerning the effects of ACE inhibitors and Ang II antagonists. Losartan at

a dose of 15 mg/kg had no effect on cardiac hypertrophy induced by coronary artery ligation, whereas in the same model captopril significantly regressed the hypertrophied heart (360). On the other hand, the increased left and right ventricular weights were significantly depressed by losartan but only moderately attenuated by enalapril in the volume overload model (322). A clinical study showed that losartan and enalapril were of comparable efficacy and tolerability in moderate or severe congestive heart failure (361). Losartan had significant advantages, with respect to long duration of action, oral absorption and no Ang II agonist activity. The improved tolerability of losartan in heart failure is not seen for ACE inhibitors due to development of cough caused by effects of bradykinin and prostaglandin. Some reports have indicated that about 10 to 15% of patients on ACE therapy have to have it discontinued due to bradykinin-related cough (362). On the other hand, ACE inhibitors also show some advantage over the Ang II blockade. One of the advantages of therapy with ACE inhibitors compared with that with Ang II receptor blockers is it increases the circulating Ang II level which is known to exert a positive inotropic effect on the myocardium (325). The most important advantage is that ACE inhibitors significantly reduce mortality and delay the development of heart failure, however, no such evidence for Ang II blockers is available yet in the literature. Likewise, in contrast of different types of ACE inhibitors (Table 4) (212, 272, 275, 277, 280, 303, 363-365), a great deal concerning the time and duration of treatment of myocardial infarction with Ang II antagonists needs to be discovered.

In view of the literature cited above, it is evident that Ang II influences heart function

Table 4: Use of various ACE inhibitors for the treatment of experimentally-induced myocardial infarction

Authors & Reference #	Animal	ACE Inhibitor	Start Treatment	Duration of Treatment	Changes of Infarct Size
Wijingaarden et al. (363)	rat	spirapril	immediately	6 weeks	↔
Fornes et al. (283)	rat	trandolapril	7 days after surgery	1 year	↔
Ertl et al. (275)	dog	SQ14225	30min-6 hr after surgery	6 hr	↓
van Wijingaarden et al. (264)	rat	captopril	before surgery	8 weeks	↔
Litwin et al. (272)	rat	captopril	immediately after surgery	21 days	↔
van Krimpen et al. (212)	rat	captopril	immediately	7 days and 21 days	↔
Wollert et al. (303)	rat	lisinopril	6-8 days after surgery	7 days, 6 weeks & 1 year	↔
Hock et al. (280)	rat	enalapril	1 min after surgery	24 hr	↔
Sweet et al. (365)	rat	enalapril	7 days after surgery	1 year	↔
Liang et al. (277)	dog	teprotide	40 min after surgery	10-40 min	↔

by effecting cardiac contraction, myocyte growth, cardiac matrix and cardiac metabolism. In this article, we have also attempted to review the influence of ACE inhibitors on different types of cardiac hypertrophy and heart failure. Although existing results are controversial, ACE inhibitors in general have been shown to exert beneficial effects on cardiac function in myocardial infarction, cardiac hypertrophy due to volume or pressure overload as well as heart failure due to pacing and cardiomyopathy. The possible mechanisms of the effects of ACE inhibitors may include reduction in both circulating and local RAS, scavenging of free radicals, improvement of energy metabolism, modification of the autonomic nervous system and increased concentration of bradykinin. More importantly, ACE inhibitors may improve cardiac function by remodeling the cell membranes, Ca^{2+} mobilization and attenuating the shift in myosin isozymes; however, the experimental evidence concerning the subcellular effects of any ACE inhibitor in the failing heart is lacking.

II. STATEMENT OF THE PROBLEM AND HYPOTHESIS TO BE TESTED

Although myocardial infarction is known to result in cardiac remodeling and subsequent heart failure, the exact mechanisms of these pathophysiological changes are poorly understood. Since SR Ca^{2+}-transport and SL Na^{+}-Ca^{2+} exchange activities are depressed in failing hearts subsequent to myocardial infarction in rats, it is likely that such changes in SR and SL membranes associated with Ca^{2+}-handling abnormalities in cardiomyocytes result in cardiac dysfunction in the failing heart. However, no information regarding the intracellular levels of Ca^{2+} in the cardiomyocytes from failing hearts due to myocardial infarction under resting and depolarizing conditions is available in literature. Since extracellular ATP plays a crucial role in modulating the intracellular level of Ca^{2+} in cardiomyocytes, it is possible that the responsiveness of cardiomyocytes to ATP is altered in heart failure. Accordingly, it is proposed to examine the intracellular levels of Ca^{2+} in cardiomyocytes from failing hearts due to myocardial infarction under resting and KCl-induced depolarizing conditions. In addition the responsiveness of sham control and failing cardiomyocytes as well as isolated heart preparations to ATP will be tested to establish the significance of Ca^{2+}-handling abnormalities in the failing hearts.

Several investigators have now identified that defects in SR mechanisms from different types of failing hearts are due to changes in the expression of genes specific for the SR proteins; however, no such information concerning abnormalities in SL Na^{+}-K^{+} ATPase or SL Na^{+}-dependent Ca^{2+} uptake is available in the literature. Furthermore, the relationship

among SL and SR protein contents and levels of mRNA specific for both SL and SR proteins in the failing hearts due to myocardial infarction has not been examined previously. In view of the marked alterations in SR Ca^{2+}-pump, SL Na^{+}-pump and SL Na^{+}-Ca^{2+} exchange activities in the failing heart subsequent to myocardial infarction, it is hypothesized that there occurs a remodeling of cardiac membranes due to changes in cardiac gene expression specific for SR and SL membranes during the development of heart failure. It is therefore proposed to examine mRNA levels for SR Ca^{2+}-pump, Ca^{2+}-release channels and phospholamban as well as for SL Na^{+}-K^{+} ATPase and Na^{+}-Ca^{2+} exchanger in failing hearts due to myocardial infarction. The biochemical activities as well as contents of their proteins will also be measured to seek relationships.

Although various ACE inhibitors are known to exert beneficial effects in the failing heart, no such information regarding a new long acting ACE inhibitor, imidapril, is available in the literature. We believe that the beneficial actions of ACE inhibitors on cardiac function in heart failure are associated with their ability to prevent membrane remodeling in the failing heart. Accordingly, it is proposed to investigate the effect of imidapril treatment on heart dysfunction subsequent of myocardial infarction in rats. Changes in Ca^{2+}-handling by cardiomyocytes as well as biochemical activities, protein contents and mRNA levels specific for SR and SL membranes will also be monitored upon treating the myocardial infarcted animals with or without imidapril. It is hoped that this study will provide new and valuable information regarding the pathophysiology of heart dysfunction due to myocardial infarction and will lend further support to the concept of subcellular remodeling during the development of heart failure.

III. METHODS

1. Experimental model

Myocardial infarction was produced in male Sprague-Dawley rats (175-200 g) by occlusion of the left coronary artery as described by Afzal and Dhalla (90). After the animals were anesthetized with 1-5% Isoflurane in 2L O_2, the skin was incised along the left sternal border, the third and fourth rib were cut open, and retractor was inserted. The heart was exposed through the left thoracotomy and the pericardial sac was gently torn. The left coronary artery was ligated 2-3 mm from its origin with a suture of 6-0 silk, and the heart was repositioned within the chest. The air in the chest was taken out by a 10 ml syringe with a plastic pipe just before closing the wound by a purse-string suture. Left coronary artery occlusion was ascertained by paling of the ventricle distal to the suture. The mortality of all animals operated on in this fashion was about 30% within 48 hr. Sham-operated animals were treated in the same way except that the coronary suture was not tied. Animals were allowed to recover in an incubator with an oxygen supply for 6-12 hr.

2. Imidapril treatment

All rats received standard care, kept at a 12 hr day/night cycle, and were fed rat chow and water *ad libitum*. The animals were randomly assigned to 4 groups: Sham control; left coronary artery ligated (MI); Sham and imidapril (IMP) treatment (Sham + IMP), ligated plus imidapril treatment (MI + IMP). Imidapril (1 mg/kg/day for 4 weeks) was given orally by a gastric tube starting at 21 days after the operation. All animals were used at 7 weeks after

the surgery for hemodynamic assessment or biochemical investigation. It should be mentioned that imidapril has been shown to be a long acting ACE inhibitor and the dose of 1 mg/kg/day used for treatment in this study has been reported to be effective in preventing hypertension and reducing mortality due to small coronary artery disease as well as due to myocardial infarction in animals (366-379). This agent has also been shown to exert beneficial effects in patients with heart failure (380). Imidapril was kindly supplied by Tanabe Seiyaku Co., Osaka, Japan.

3. Hemodynamic studies

The animals were anesthetized with an intraperitoneal injection of the mixed ketalean (60 mg/kg) and xylazine (10 mg/kg). The right carotid artery was exposed and a cannula with a microtip pressure transducer (model SPR-249, Millar Instruments, Houston, TX) was introduced through a proximal arteriotomy (381). The arterial blood pressure was measured at this point. The catheter was advanced carefully through the lumen of the carotid artery until it entered the left ventricle. The catheter was secured with a silk ligature around the artery, and the readings were taken from a computer program (AcqKnowledge for Windows 3.0, Harvard Apparatus, Montreal, Canada). In some experiments rat jugular vein was isolated and a cannula was inserted for injecting ATP. The left ventricular systolic pressure, left ventricular end diastolic pressure (LVEDP), heart rate, rate of contraction (+dP/dt) and rate of relaxation (-dP/dt) were measured in these anesthetized animals. At the end of the hemodynamic measurements, the hearts were removed and the right and left

ventricles as well as scar tissue were dissected and weighed. It should be noted that the experimental animals with large infarct size (30-45%) of the left ventricle were employed in this study.

4. Isolation of single cardiomyocytes

Coronary artery ligated or sham operated rats with or without imidapril treatment were injected with heparin intravenously (1000 unit/100 g) 10 min before use, anesthetizing with ketalean (60 mg/kg) combined with xylazine (10 mg/kg) given intraperitoneally. The heart was moved rapidly and washed in ice cold perfusion buffer containing (in mM): NaCl 90, KCl 10, KH_2PO_4 1.2, $MgSO_4$ 5.0, $NaHCO_3$ 15, taurine 30, glucose 10, then oxygenated with 95% O_2 and 5% CO_2 (pH 7.4). The heart was cannulated on the Langendorff apparatus via the aorta, perfused in a non-circulating manner with Ca^{2+}-free buffer gassed with 95% O_2 and 5% CO_2 for 10 min. It was then perfused with the medium containing 0.1% collagenase (CLS2, Worthington Biochemical Corp., Freehold, NJ, USA), 50 μM $CaCl_2$ and 0.1% bovine serum albumin for 30 min. The heart was taken off the cannula, the septum and the left ventricular free wall (without scar tissue) were dissected out, cut into small pieces and subjected to another 30 min digestion in the fresh collagenase solution at 37°C in a shaking water bath. Cell suspensions were collected every 10 min and washed with the above perfusion buffer containing increasing amounts of 50 μM, 250 μM, 500 μM, 750 μM and 1 mM Ca^{2+} at one min intervals. The rod shaped cells obtained by this method (382, 383) were more than 80%.

5. Intracellular $[Ca^{2+}]$ measurements

The method for measuring $[Ca^{2+}]_i$ by using the Fura-2/AM technique was the same as described earlier (382, 383). Briefly, freshly isolated cardiomyocytes were incubated with 5 μM Fura-2 acetomethyl ester (Fura-2/AM, Molecular Probes, Inc., Eugene, OR, USA) in a buffer containing (in mM): NaCl 120, KCl 4.74, KH_2PO_4 1.2, Mg SO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 1.0, glucose 10 and 1% bovine serum albumin oxygenated with 95% O_2 and 5% CO_2 (pH 7.4) at 37°C for 40 min. The cells were stimulated with 50 μM ATP or 30 mM KCl. Some cells were preincubated with verapamil, ryanodine or an ATP receptor blocker, Cibacron Blue, for 10-30 min at room temperature and then stimulated with ATP. Fluorescent signals were recorded with a spectroflurometer (SLM DMX-1100) at two wavelengths of excitation (340 nm and 380 nm) and one wavelength of emission (510 nm). Fluorescent signals obtained at 340 nm and 380 nm and their ratio were stored in a computer program (SLM 8100). The $[Ca^{2+}]_i$ levels at rest as well as the maximal increase evoked by agonists were calculated according to the formula: $[Ca^{2+}]_i = 224 \times R - R_{min}/R_{max}$ -RX Sf2/Sb2 (384). R_{max} and R_{min} values were determined by inclusion of 40 μl Triton-100 (10%) and 40 μl EGTA (400 mM).

6. ATP-receptor binding assay

In this experiment we used the membrane preparation which sediments at low centrifugal forces (385); this preparation was suspended in 250 mM sucrose, 50 mM Tris-HCl, pH 7.4, at a concentration of 3-5 mg/ml, frozen in liquid nitrogen, stored at -80°C, and

used within 2-3 weeks. 30-50 μg membrane protein was incubated in 0.5 ml medium containing various concentration of ATPγS (0.5 to 10 nM) and 50 mM Tris-HCl, pH 7.5, at 37°C for 30 min as described elsewhere (386). The reaction was terminated by vacuum filtration over wet Whatman filters (GF/B), using a cell harvester (M-24R, Brandel, Gaithersburg, MD, USA). The filters were washed three times with 6 ml of ice cold water and the radioactivity was counted in a Beckman scintillation counter. The binding was determined in the absence (total) and presence (non-specific) of 4 mM ATP; the specific binding was calculated by subtracting non-specific binding from the total binding. For avoiding the possible artefacts, the binding of the radioligand to the GF/B filters was also checked in the absence of membrane protein from the assay tubes.

7. Preparation of cardiac SL membrane

Cardiac SL membrane was isolated from sham control and experimental animals with or without imidapril treatment according to a modified method of Pitts (387). The frozen left ventricle without scar tissue from 3 to 4 hearts in each group was allowed to thaw in an ice cold buffer containing 0.6 M sucrose, 10 mM imidazole-HCl, pH 7.0 (3.5 ml per g heart tissue). Then the tissue was chopped with scissors and homogenized with a Polytron PT-20 (5 x 20 sec, 5 min interval). The resulting homogenate was centrifuged at 12,000 g for 30 min at 4°C and the pellet was discarded. After diluting (5 ml/g tissue) with 140 mM KCl, 20 mM (N-morpholino) propanesulfonic acid (MOPS), pH 7.4 (KCl-MOPS buffer), the supernatant was centrifuged at 95,000 g for 60 min. This pellet was suspended in KCl-

MOPS buffer and layered over a 30% sucrose solution containing 0.3 M KCl, 50 mM $Na_4P_2O_7$ and 0.1 M Tris-HCl, pH 8.3. After centrifugation at 95,000 g for 90 min (utilizing a Beckman swinging bucket rotor 42.1), the band at the sucrose-buffer interface was taken and diluted with 3 volume of KCl-MOPS buffer. The final centrifugation at 95,000 g for 30 min resulted in a pellet rich in SL which was resuspended in 0.25 M sucrose, 10 mM histidine, pH 7.0. Samples were then stored at -70°C in aliquots before carrying out biochemical studies. This method of isolating cardiac SL has been employed in our laboratory previously (89, 388).

8. Measurement of total Na^+-K^+ ATPase activity

Estimation of Na^+-K^+ ATPase activity was carried out by a previously described method (388) with some modification. Briefly, 10 μg of SL membrane was preincubated at 37°C with 1.0 mM ethylene glycol-bis (β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA)-Tris, pH 7.4, 5 mM NaN_3, 6 mM $MgCl_2$, 100 mM NaCl, and 10 mM KCl, 2.5 mM phosphoenolpyruvate (PEP), and 10 IU/ml pyruvate kinase. PEP and pyruvate kinase were used as ATP-regenerating system to maintain the concentration of ATP in the incubation medium. The reaction was started by the addition of 0.025 ml 80 mM ATP, pH 7.4, and terminated after 10 min with 0.5 ml ice-cold 12% trichloroacetic acid. The liberated phosphate was measured by the method of Taussky and Shorr (389). Mg^{2+} ATPase activity was estimated as the difference between the activities with and without Mg^{2+} in the absence of Na^+ and K^+ in the medium. All measurements were carried out in duplicate.

9. SL Na^+-dependent Ca^{2+}-uptake measurement

The Na^+-dependent Ca^{2+}-uptake in SL vesicles was measured by a method described elsewhere (89). In short, 5 µl of SL vesicle (1.5 mg/ml; 7.5 µg protein/tube) preloaded with NaCl-MOPS buffer at 37°C for 30 min were rapidly diluted 50 times with Ca^{2+}-uptake medium containing 140 mM KCl, 20 mM MOPS, 0.4 µM valinomycin, 0.3 μC_i $^{45}Ca^{2+}$, pH 7.4. After the appropriate time span, the reaction was stopped by the addition of ice-cold 0.03 ml stopping solution containing (in mM) 140 KCl, 1 $LaCl_3$ and 20 MOPS, pH 7.4. Radioactivity for the total Ca^{2+}-uptake activity was measured with a Beckman LS 1701 counter. In parallel with these samples, non-specific Ca^{2+}-uptake was measured by placing the Na^+-loaded SL vesicles in Ca^{2+}-uptake medium, which contained 140 mM NaCl instead of KCl. The Na^+-dependent Ca^{2+}-uptake activity was calculated by subtracting the non-specific Ca^{2+}-uptake value from the total Ca^{2+}-uptake activity.

10. SR membrane isolation

Membrane fraction enriched with SR was isolated according the method described by Ganguly et al. (390). Briefly, viable nonischemic left ventricular tissue was homogenized in a Waring blender in a medium containing (in mM) 10 $NaHCO_3$, 5 NaN_3, and 15 Tris-HCl (pH 6.8) at the medium speed for 45s. The homogenate was centrifuged at 10,000 g for 20 min, the pellet was discarded, and the supernatant was centrifuged at 40,000 g for 30 min. The pellet was suspended in 0.6 M KCl and 20 mM Tris-HCl (pH 6.8) to solubilize the contractile proteins and again centrifuged at 40,000 g for 45 min. The final pellet was

washed and suspended in 0.25 M sucrose and 20 mM Tris-HCl (pH 6.8) and stored at -70°C.

11. Determination of SR Ca^{2+}-uptake

Ca^{2+}-uptake was determined using the Millipore filtration technique (90). The membrane (0.05 mg/ml) was incubated in presence of 100 mM KCl, 20 mM Tris-HCl (pH 6.8), 5 mM $MgCl_2$, 5 mM K-oxalate and 5 mM NaN_3. The desired concentration of ^{45}Ca was 10 μM. The concentration of free Ca^{2+} in solutions was buffeted by EGTA and calculated with a program developed by Fabiato (391). The reaction was started with 5 mM ATP after 5 min and a 0.1 ml sample was filtered through a Millipore filter (pore size 45 μm) and immediately washed twice with 3 ml ice-cold buffer. The filters were dried and then counted for radioactivity for total Ca^{2+}-uptake by using a standard liquid scintillation counting technique. Appropriate blanks in the absence of ATP were subtracted from the total Ca^{2+}-uptake to calculate the ATP-dependent Ca^{2+}-uptake.

12. Determination of SR Ca^{2+} ATPase activity

Total ($Mg^+ + Ca^{2+}$) and basal (Mg^+)-ATPase activities (392) were determined in an incubation medium similar to that used for the SR Ca^{2+}-uptake assay described above except that when total ATPase was measured, nonradioactive $CaCl_2$ (final 10 μM free Ca^{2+}) was used and when basal ATPase was measured, Ca^{2+} was omitted and 0.2 mM EGTA was added. The reaction was started by the addition of 5 mM Tris-ATP after a 3 min preincubation with 50 μg of membrane. The reaction was terminated by 12% ice-cold

trichloroacetic acid. Inorganic phosphate liberated in the protein-free filtrate was assayed. The Ca^{2+}-stimulated Mg^{+}-dependent ATPase activity is the difference between the total and basal ATPase values.

13. Assay for ^{3}[H]-ryanodine binding

Ryanodine binding was determined as described by Zucchi et al. (393) with minor changes. Membranes (0.5 mg/ml) enriched in SR were incubated at 37°C for 60 min in a buffered medium (final volume 1 ml) containing 25 mM imidazole (pH 7.4 at 37°C), 1 M KCl, 0.2 to 50 nM ^{3}[H]-ryanodine (6 Ci/mM), 0.2 M Tris-HCl. Free Ca^{2+} concentration was 20 μM. The nonspecific binding was determined in the presence of 100 μM ryanodine. The binding reaction was terminated by filtration through cellulose nitrate filters with pore size of 0.45 μM pre-soaked in 25 mM imidazole (pH 7.4) and 1 M KCl (washing buffer). The filters were washed with 3 ml aliquot of washing buffer and then overnight in 10 ml scintillation fluid. Specific binding was calculated by subtracting nonspecific binding from total binding values.

14. SDS-PAGE and Western blot assay

SL and SR membranes were diluted to 2.0 mg/ml concentration with 0.25 M sucrose and 10 mM histidine. The relative amounts of SL Na^{+}-K^{+} ATPase and Na^{+}-Ca^{2+} exchanger as well as SR Ca^{2+} ATPase, ryanodine receptor and phospholamban proteins were determined by running 6-10% mini gel with a 4% staking gel of sodium dodecyl

sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (394). The SL and SR vesicles (2 mg/ml) were added to the SDS-PAGE loading buffer containing 0.1 M Tris-HCl (pH 6.8), 15% (w/v) SDS, 15% glycerol, 8% β-mercaptoethanol and 0.002% bromophenol blue. The ratio of SL or SR membranes and the loading buffer was 1:1. The SDS-PAGE was carried out at 200 V for 1 hr. The proteins, separated by SDS-PAGE, were then electroblotted to PVDF membrane (Millipore Corporation, Bedford, MA, USA) in transfer buffer containing 25 mM Tris-HCl, 192 mM glycine and 4% methanol (v/v) at 0.5 mA. The transferred membranes were shaken overnight in blocking buffer (TBS, 10 mM Tris, 150 mM NaCl and 5% fat-free powdered milk) at 4°C, and then incubated for 1 hr at room temperature in monoclonal anti-Na^+-K^+ ATPase antibodies (α_1-subunit mouse IgG (1:10,000), polyclonal subunits rabbit IgG α_2 (1:2,000) and β_1 (1:2,000) antibodies (Upstate Biotechnology, Lake Placid, NY, USA), polyclonal anti Na^+-Ca^{2+} exchanger antibody (1:1,500 Swant Swiss Antibodies, Switzerland), mouse monoclonal anti-SR Ca^{2+}-ATPase antibody (1:2,000 Affinity Bioreagents Inc., Golden, CO, USA), mouse anti-SR phospholamban antibody (1:2,000 Upstate Biotechnology, Lake Placid, NY, USA) and anti SR-ryanodine receptor antibody (1:1,800 Upstate Biotechnology, Lake Placid, NY, USA) (in TBS buffer containing 3-5% fat-free powdered milk and 1% Tween-20). The membranes were subsequently incubated 1 hr with second antibody (biotinylated anti-rabbit IgG antibody (1:1,000 for α_1-subunit, 1:3,000 for α_2- and β_1-subunits) and anti-mouse IgG antibody (1:3,000) Amersham Corporation, Arlington Heights, IL, USA). The membranes were incubated with strepdavidin conjugated

horseradish peroxidase (1:5,000; Amersham Corporation, Arlington Heights, IL, USA) in TBST for 30 min at room temperature. The blots were rinsed in the TBST buffer 3 times (15 min each time) between each of the preceding steps. For chemiluminescent detection, the membrane was dipped into the ECL Kit, (Amersham Corporation, Arlington Heights, IL, USA) and the chemilumigrams were developed on ECL-Hyperfilm (Amersham Corporation) to visualize Na^+-K^+ ATPase, Na^+-Ca^{2+} exchanger, SR Ca^{2+} ATPase, ryanodine receptor and phospholamban bands. The bands were analyzed by the model GS-670 Imaging Densitometer (Bio-Rad Company, Mississauga, ON, Canada) with the Image Analysis Software Version 1.0 and was expressed in relation to control values.

15. Northern blot analysis and molecular probes

Total cellular RNA was isolated from the frozen left ventricles by the method of acid guanidinium thiocyanate/phenol/chloroform extraction (395). The final RNA pellet was resuspended in sterile distilled water containing 0.1% DEPC and stored at -70°C. Twenty micrograms of total RNA was denatured at 65°C for 10 min, size fractioned on a 1.2% agarose gel containing 1 M formaldehyde, blotted onto a nytran membrane (Schleicher & Schuell, Keene, NH, USA), UV-cross-linked, and hybridized to random primed cDNA or oligonucleotide probes. The membranes were washed with 1 x standard saline citrate and 0.1% SDS at room temperature for 20 min, exposed to Kodak X-Omat-AR film using an intensive screen at -70°C. After autoradiography, individual mRNA bands were quantitated using a GS-670 (Bio-Rad Company, Mississauga, ON, Canada). The optical density of each

of the bands was divided by the GAPDH or 18S optical density. The relative level of these messages correlated against the GAPDH value in each sample was calculated as percentage of the mean value of the corresponding message level in the sham control group. The following cDNA and oligonucleotide probes were used for Northern blot analysis: (a) SERCA2: a 0.762 kb cDNA fragment of the rabbit cardiac Ca^{2+}-ATPase (courtesy of Dr. A.K. Grover, McMaster University, Hamilton, ON, Canada); (b) Phospholamban: a 0.153 kb cDNA fragment of the rabbit cardiac phospholamban (courtesy of Dr. D.H. MacLennan, University of Toronto, Toronto, Canada); (c) Ryanodine receptor: a 2.2 kb cDNA fragment of the rabbit cardiac ryanodine receptor (Courtesy of Dr. D.H. MacLennan, University of Toronto, Toronto, Canada); (d) Calsequestrin: a 2.5 kb cDNA fragment of the rabbit cardiac calsequestrin (courtesy of Dr. A. Zilberman, University of Cincinnati, Cincinnati, USA); (e) Na^+-K^+ exchanger: a 1.0 kb cDNA fragment of the dog heart Na^+-Ca^{2+} exchanger (courtesy of Dr. K.D. Philipson, Los Angeles, USA); (f) Na^+-K^+ ATPase isoforms: 0.332, 0.381, 0.278 and 0.271 kb cDNA fragments of the rat brain Na^+-K^+ ATPase isoforms (α_1, α_2, α_3 and β_1) (American Type Culture Collection, Rockville, MD, USA); (g) 18S: a 24 base oligonucleotide probe of rat 18S ribosomal RNA; (h) Glyceraldehyde-3-phosphate dehydrogenase (GAPDH): a 1.2 kb cDNA fragment of the human GAPDH (American Type Culture Collection, Rockville, MD, USA). GAPDH and 18S ribosomal RNA levels were used as an internal standard for the variations in same loading and blotting efficiency of RNA.

16. Statistical analysis

Results are presented as mean ● SE. For multiple comparisons, the data were subjected to analysis of variance (ANOVA) and Duncan's multiple-range test. The difference between the control and the experimental group values were further verified by using Student's *t*-test. Probability (P) value of less than 0.05 was considered statistically significant.

IV. RESULTS

1. Heart function and ATP-induced alterations

The first series of experiments was undertaken to assess the status of left ventricular function in rats with coronary occlusion for a period of 7 weeks. The results in Table 5 indicate that left and right ventricular wt. were significantly ($P < 0.05$) increased in the infarcted animals. Although no changes in the heart rate or LVSP were evident, the LVEDP was greatly elevated in the infarcted animals. Furthermore, both +dP/dt and -dP/dt were markedly depressed in rats with 7 weeks of myocardial infarction. In order to test the effect of IMP on heart function, sham control and 3 weeks infarcted animals were treated with the drug (1 mg/kg, daily) for a period of 4 weeks. We selected 3 weeks infarcted animals for treatment with IMP because the infarct is completely healed 3 weeks after occlusion of the coronary artery in rats (381). The selection of the dose of IMP for treatment was based on our previous studies showing beneficial effect of this agent during early phases of myocardial infarction (379). The data in Table 5 indicate that treatment of sham control animals, unlike infarcted animals, with IMP significantly ($P < 0.05$) reduced the body wt. and left ventricular wt. On the other hand, the right ventricular wt. was significantly depressed in the infarcted animals, unlike the sham controls. The scar wt. in the infarcted animals was not altered by IMP treatment. While IMP treatment did not affect the hemodynamic parameters in the sham control animals, this drug was found to markedly attenuate the elevated LVEDP as well as depressed +dP/dt and -dP/dt in the infarcted animals (Table 5).

Since ATP released from the nerve endings is known to modulate heart function

Table 5: General and hemodynamic characteristics of myocardial infarcted rats with or without imidapril treatment for 4 weeks starting at 3 weeks after coronary occlusion

	Sham	Sham + IMP	MI	MI + IMP
Body wt (g)	522 ± 17	452 ± 8.2*	487 ± 11	470 ± 18
Left ventricular wt (mg)	902 ± 22	800 ± 30*	1166 ± 30*	1108 ± 28*
Right ventricular wt (mg)	263 ± 12	270 ± 14	330 ± 12*	283 ± 10#
Scar wt (mg)	ND	ND	241 ± 25	247 ± 18
Heart rate (beats/min)	284 ± 13	251 ± 17	284 ± 23	294 ± 10
LVEDP (mm Hg)	3.4 ± 0.4	3.2 ± 0.5	14.9 ± 0.8*	4.3 ± 0.4#
LVSP (mm Hg)	140 ± 7.2	145 ± 6.9	130 ± 8.8	140 ± 12
+dP/dt (mm Hg/sec)	4750 ± 417	4470 ± 364	1820 ± 170*	3864 ± 340#
-dP/dt (mm Hg/sec)	4880 ± 446	4656 ± 206	1842 ± 204*	4052 ± 410#

Values are mean ± S.E. of 10 animals in each group. ND: not detectable; MI: myocardial infarcted; LVSP: left ventricular systolic pressure; LVEDP: left ventricular end diastolic pressure; +dP/dt: rate of contraction; -dP/dt: rate of relaxation. Left ventricular wt. for MI and MI+IMP groups includes scar tissue. Imidapril (IMP) was given orally (1 mg/kg, daily). *$P < 0.05$ compared with sham group. #$P < 0.05$ compared with MI group.

(396-401), the responses of the failing heart to ATP were examined by injecting ATP into the anesthetized control and 7 weeks infarcted animals and monitoring the changes in heart function at 1 or 2 min after the injection. The results in Figs. 1 and 2 show that the ATP-induced increase in LVDP, +dP/dt or -dP/dt was markedly reduced in the infarcted animals in comparison to the control values. In addition, ATP was found to exert no appreciable effect on the heart rate in both control and infarcted animals. Treatment of infarcted animals with IMP was observed to attenuate the ATP-induced depressions in LVDP, +dP/dt and -dP/dt without effect on the heart rate (Figs. 1 and 2). These results indicate that the positive inotropic responses to ATP are depressed in animals with heart failure and the treatment of infarcted animals with IMP produces a beneficial effect in this regard.

2. ATP-induced changes in intracellular Ca^{2+}

Because the positive inotropic effect of ATP is considered to be due to its ability to increase the intracellular concentration of free Ca^{2+} (402-404), it is possible that the observed ATP-induced changes in heart function in the infarcted animals with or without imidapril treatment may be due to alterations in its effect on $[Ca^{2+}]_i$. Accordingly, we examined the actions of ATP on $[Ca^{2+}]_i$ by employing cardiomyocyte preparations from sham control and infarcted animals. The results in Fig. 3 and Table 6 show that the basal levels of $[Ca^{2+}]_i$ in the left ventricular cardiomyocytes from the sham and infarcted animals with or without IMP treatment were not significantly different from each other. ATP produced a slow increase in $[Ca^{2+}]_i$ in control cardiomyocytes which reached maximal at about 100 sec and declined

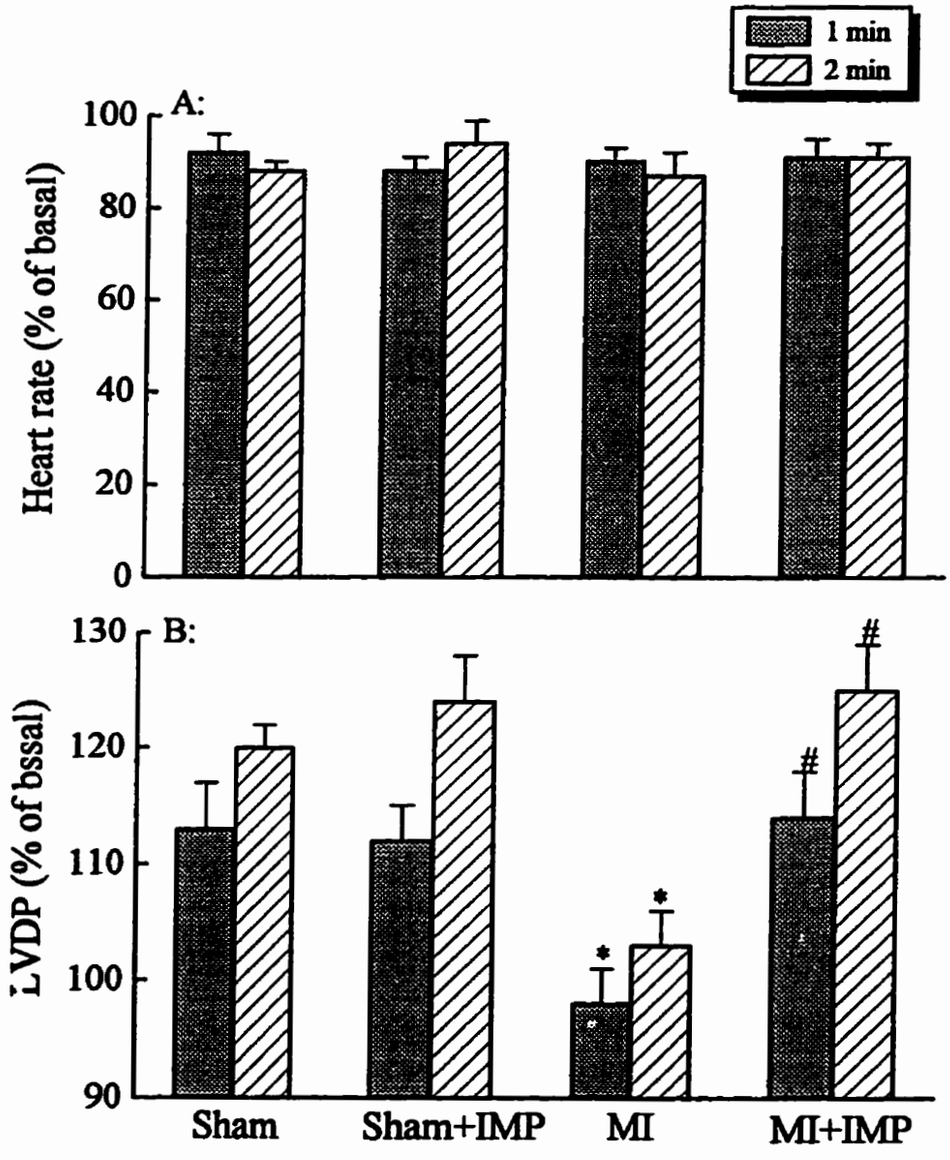

Figure 1 Changes in heart rate and the left ventricular developed pressure (LVDP) due to ATP administration in sham and 3 weeks infarcted (MI) rats with or without imidapril (IMP) treatment (1 mg/kg/day; orally) for 4 weeks. Changes shown here were monitored at 1 and 2 min of injecting ATP (0.62 μg/kg; i.v.) and are presented as % of the basal value recorded before the ATP injection in each group. The basal values for these parameters were similar to those given in Table 1. The values are mean ± SE from 6 animals in each group. *P < 0.05 in comparison to the respective sham control. #P < 0.05 in comparison to the respective value for the MI group.

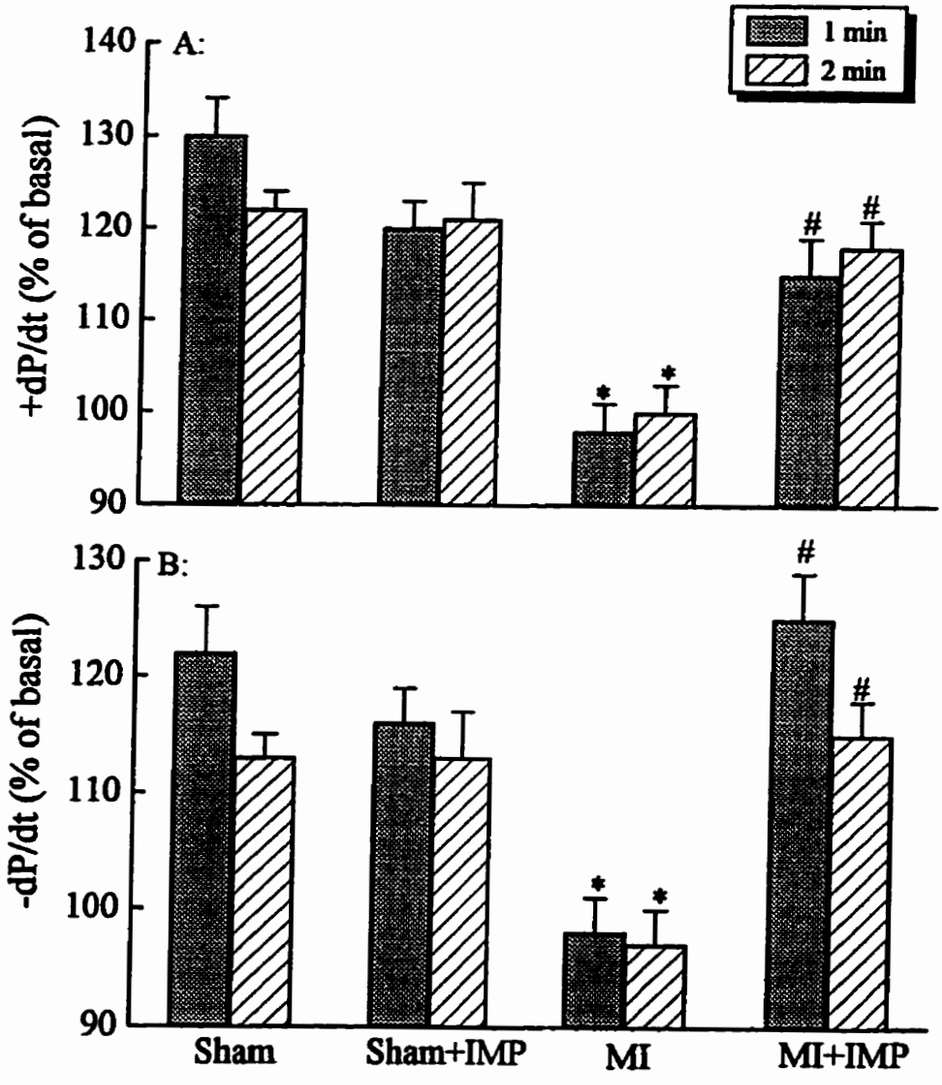

Figure 2 Changes in the left ventricular rate of contraction (+dP/dt) and rate of relaxation (-dP/dt) due to ATP administration in sham and 3 weeks infarcted (MI) rats with or without imidapril (IMP) treatment (1 mg/kg/day; orally) for 4 weeks. Changes shown here were monitored at 1 and 2 min of injecting ATP (0.62 μg/kg; i.v.) and are presented as % of the basal value recorded before the ATP injection in each group. Basal values for these parameters were similar to those given in Table 1. The values are mean ± SE from 6 animals in each group. *$P < 0.05$ in comparison to the respective sham control; #$P < 0.05$ in comparison to the respective MI group.

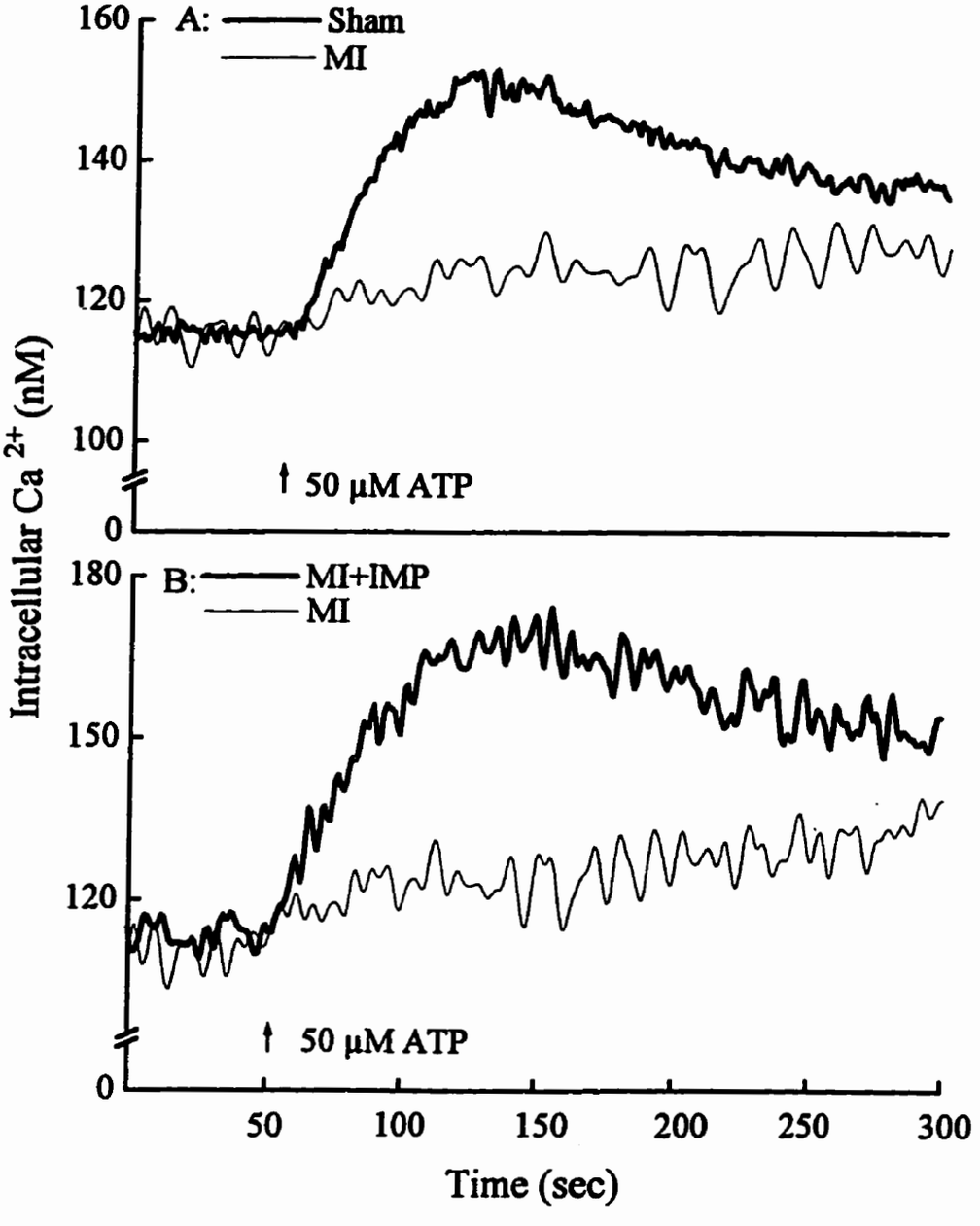

Figure 3 Typical tracings of changes in the intracellular free Ca^{2+} due to ATP in the left ventricular cardiomyocytes from sham and 3 weeks infarcted (MI) rats with or without imidapril (IMP) treatment (1 mg/kg/day; orally) for 4 weeks.

Table 6: Influence of ATP on intracellular concentration of Ca^{2+} in left ventricular myocytes of myocardial infarcted rats with or without imidapril treatment for 4 weeks starting at 3 weeks after coronary occlusion

	Basal $[Ca^{2+}]_i$ (nM)	Increase in $[Ca^{2+}]_i$ after 100 sec of ATP addition (% of basal value)	Increase in $[Ca^{2+}]_i$ after 250 sec of ATP addition (% of basal value)
Sham	120 ± 10	33 ± 2.3	23 ± 1.7
Sham + IMP	125 ± 12	32 ± 4.4	25 ± 2.1
MI	118 ± 6	12 ± 2.1*	20 ± 2.6
MI + IMP	116 ± 5	32 ± 2.8#	27 ± 3.1

Values are means ± S.E. of 5 experiments in each group. MI: myocardial infarcted. Imidapril (IMP) was given orally (1 mg/kg, daily). The concentration of ATP was 50 μM. *$P < 0.05$ compared with sham control. #$P < 0.05$ compared with MI group.

thereafter. On the other hand, the ATP-induced increase in $[Ca^{2+}]_i$ in infarcted preparations did not show any peak and thus showed reduction in the elevation of $[Ca^{2+}]_i$ by ATP during early phases. The data in Table 6 show a significant depression in the ATP-induced increase in $[Ca^{2+}]_i$ in the infarcted preparations, when measured at 100 sec, but not at 250 sec of exposing cardiomyocytes to ATP. Treatment of infarcted animals with IMP attenuated the ATP-induced the observed changes in $[Ca^{2+}]_i$; IMP treatment produced no effect on the ATP-induced increase in $[Ca^{2+}]_i$ in the sham control preparations (Table 6). It can be seen from Table 7 that ATP-induced increases in $[Ca^{2+}]_i$ in cardiomyocytes from sham control and infarcted animals with or without IMP treatment were markedly prevented by the presence of verapamil, a blocker of SL Ca^{2+}-channels and Cibaeron blue, a blocker of SL purinergic receptors. Although ryanodine, a blocker of Ca^{2+}-release channels in SR, also reduced the ATP-induced increase in $[Ca^{2+}]_i$ in all preparations significantly except the cardiomyocytes from untreated infarcted animals.

3. Alterations in ATP-receptors and KCl-induced changes in intracellular Ca^{2+}

In order to gain some further information regarding mechanisms of the observed changes in ATP-induced responses in failing hearts, we examined the status of ATP receptors. The results in Table 8 show no changes in the affinity ($1/K_d$) or maximal density (B_{max}) of ATP receptors in cardiac SL membranes obtained from control and infarcted animals with or without IMP treatment. The specificity of ATP-induced changes in $[Ca^{2+}]_i$ was tested upon studying the response of control and experimental cardiomyocytes to KCl.

Table 7: Effect of some inhibitors on the increase in intracellular Ca^{2+} due to ATP in left ventricular myocytes of myocardial infarcted rats with or without imidapril treatment for 4 weeks starting at 3 weeks after coronary occlusion

	Sham	Sham + IMP	MI	MI + IMP
No Drug	125 ± 4	133 ± 3	114 ± 2	130 ± 2
Verapamil	102 ± 2*	100 ± 1*	102 ± 3*	103 ± 3
Ryanodine	110 ± 4*	112 ± 3*	111 ± 3	114 ± 4*
Cibacron blue	109 ± 5*	107 ± 4*	104 ± 4*	109 ± 5*

Values are means ± S.E. of 4 experiments and are expressed as % of the basal values in each group. MI: myocardial infarcted. Imidapril (IMP) was given orally (1 mg/kg, daily). The concentrations of verapamil, ryanodine and cibacron blue were 10 μM, 10 μM and 100 μM, respectively. The cells were preincubated with drugs for 10 min before the addition of 50 μM ATP. *$P < 0.05$ compared with no drug group.

Table 8: Changes in ATP receptors in sarcolemma from left ventricle of myocardial infarcted rats with or without imidapril treatment for 4 weeks starting at 3 weeks after coronary occlusion

	K_d (nM)	B_{max} (pmol/mg)
Sham	11.9 ± 0.8	10.1 ± 0.5
Sham + IMP	10.2 ± 1.1	9.2 ± 1.0
MI	10.2 ± 0.7	9.3 ± 0.5
MI + IMP	10.4 ± 0.7	11.6 ± 0.8

Values are means ± S.E. of 4 samples in each group. MI: myocardial infarction. Imidapril (IMP) was given orally (1 mg/kg, daily). The characteristics of ATP receptors were studied by monitoring specific binding of [^{35}S]-γ-ATP at different concentrations and the data were analyzed by the Scatchard plot analysis.

The results indicate that KCl produced a rapid increase in $[Ca^{2+}]_i$ in both control and experimental cardiomyocytes; however, this increase remained lower in the infarcted preparations in comparison to the control (Fig. 4 and Table 9). Treatment of infarcted animals with IMP markedly attenuated the observed changes in KCl-induced increase in $[Ca^{2+}]_i$ but had no effect on preparations from sham control animals (Fig. 4 and Table 9). Such changes in KCl-induced increase in $[Ca^{2+}]_i$ in experimental preparations may indicate that alterations in ATP-induced response in the infarcted myocardium may be due to a general Ca^{2+}-handling defect in the failing heart.

4. SL Na^+-K^+ ATPase and Na^+-dependent Ca^{2+}-uptake

In view of the role of SL membrane in controlling Ca^{2+}-movements in cardiomyocytes, we examined changes in the SL membrane from the failing hearts by monitoring Na^+-K^+ ATPase and Na^+-Ca^{2+} exchange activities. The results in Fig. 5 indicate that the Na^+-K^+ ATPase activity was significantly depressed in SL preparations from the infarcted animals. Such a depression in the Na^+-K^+ ATPase activity was prevented upon treatment of the infarcted animals with IMP, which treatment in sham control animals did not produce any effect on the enzyme activity. It may also be noted from Fig. 5 that the Mg^{2+} ATPase activity in control membranes was not different from that in the experimental preparations with or without IMP treatment. A defect in the SL membrane is also evident from our finding that the Na^+-dependent Ca^{2+}-uptake activity was depressed in the infarcted preparations (Fig. 6). IMP treatment, which had no effect in the sham control animals, was found to attenuate the

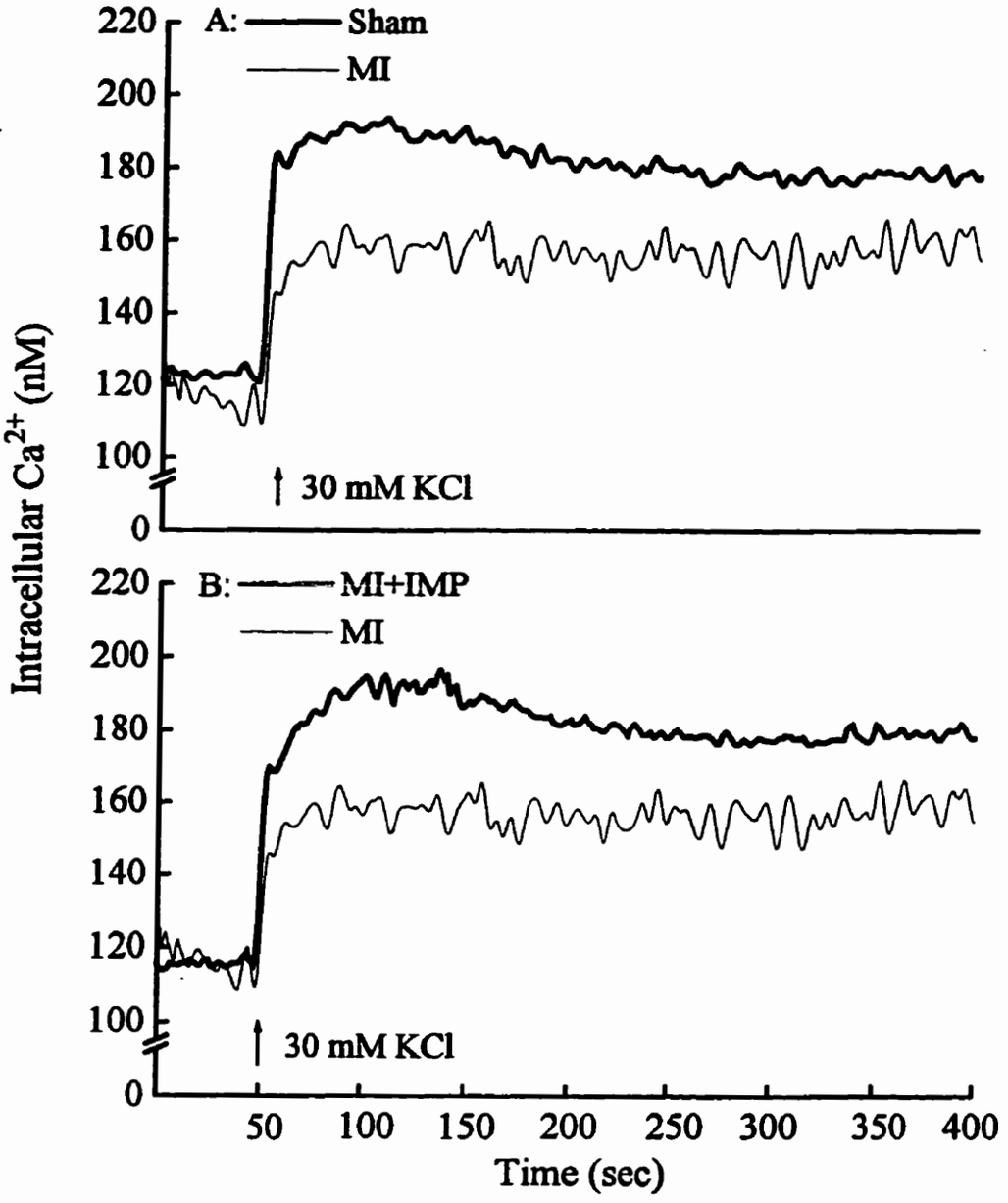

Figure 4 Typical tracings of changes in the intracellular free Ca^{2+} due to KCl in the left ventricular cardiomyocytes from sham and 3 weeks infarcted (MI) rats with or without imidapril (IMP) treatment (1 mg/kg/day; orally) for 4 weeks.

Table 9: Influence of KCl on intracellular concentration of Ca^{2+} in left ventricular myocytes of myocardial infarcted rats with or without imidapril treatment for 4 weeks starting at 3 weeks after coronary occlusion

	Basal $[Ca^{2+}]_i$ (μM)	Increase in $[Ca^{2+}]_i$ at 75 sec after KCl addition (% of basal value)	Increase in $[Ca^{2+}]_i$ at 250 sec after KCl addition (% of basal value)
Sham	122 ± 6	65 ± 3.8	57 ± 2.6
Sham + IMP	125 ± 5	64 ± 3.2	54 ± 2.5
MI	116 ± 4	29 ± 2.6*	31 ± 2.3*
MI + IMP	118 ± 5	60 ± 2.4#	51 ± 3.4#

Values are means ± S.E. of 5 experiments in each group. MI: myocardial infarcted. Imidapril (IMP) was given orally (1 mg/kg, daily). The concentration of KCl was 30 mM. *$P < 0.05$ compared with sham control. #$P < 0.05$ compared with MI group.

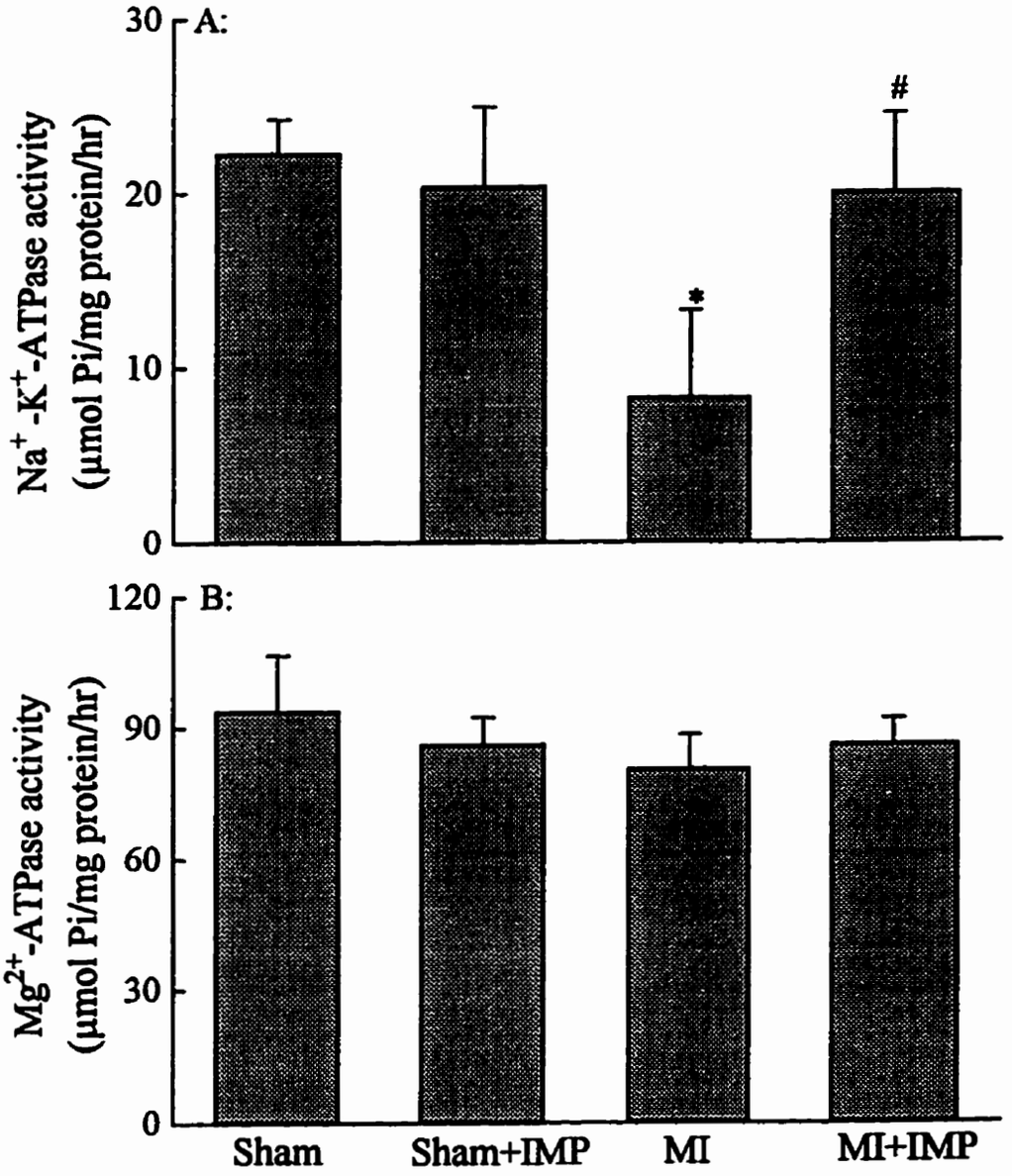

Figure 5 Sarcolemmal Na^+-K^+ ATPase and Mg^{2+} ATPase activities in left ventricles from sham and 3 weeks infarcted (MI) rats with or without imidapril (IMP) treatment for 4 weeks. ATPase activities were determined by measuring ATP hydrolysis for 10 min. IMP was given orally (1 mg/kg, daily). Each value is a mean ± SE of 5 samples in each group. $^*P < 0.05$ compared with sham control. $^{\#}P < 0.05$ compared with MI group.

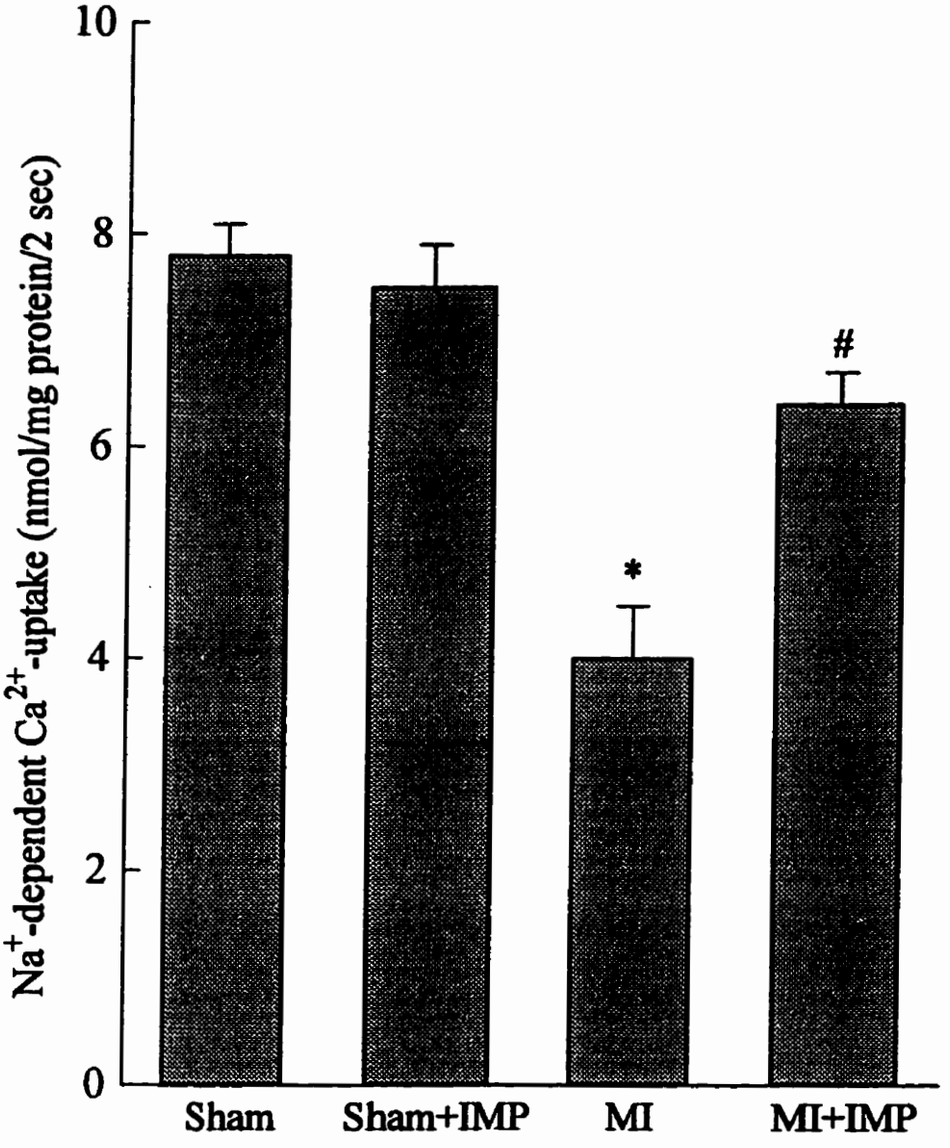

Figure 6 Sarcolemmal Na^+-dependent Ca^{2+}-uptake activity in left ventricles from sham and 3 weeks infarcted (MI) rats with or without imidapril (IMP) treatment for 4 weeks. Na^+-dependent Ca^{2+}-uptake was determined for 2 sec. IMP was given orally (1 mg/kg, daily). Values are mean ± SE of 5 samples in each group. *$P < 0.05$ compared with sham control. #$P < 0.05$ compared with MI group.

depressed Na^+-Ca^{2+} exchange activity in the infarcted animals (Fig. 6).

In order to show if the observed changes in Na^+-K^+ ATPase and Na^+-dependent Ca^{2+}-uptake activities were associated with similar changes in the SL protein content from these biochemical parameters, we obtained Western blots (Fig. 7) for these proteins by employing antibodies specific for Na^+-Ca^{2+} exchanger and different isoforms of Na^+-K^+ ATPase in control and infarcted animals with or without IMP treatment. The results in Fig. 8 indicate a dramatic decrease in the content of α_1-isoform of Na^+-K^+ ATPase in the infarcted preparation; this change was partially prevented by treatment of infarcted animals with IMP. On the other hand, a moderate depression in the Na^+-K^+ ATPase β_1-isoform content of the experimental membrane was fully prevented by IMP treatment. A marked decrease in the content of α_2-isoform and a marked increase in the content of α_3-isoform in the failing heart SL membrane were partially prevented by IMP treatment (Fig. 9). It should also be noted from Figs. 8 and 9 that treatment of sham control animals with IMP had no effect on the content of Na^+-K^+ ATPase isoforms. The depressed level of Na^+-Ca^{2+} exchange protein in the SL membrane from the failing hearts was also partially prevented by treatment of infarcted animals with IMP, which did not exert any significant effect in the sham control animals (Fig. 10).

5. SL Na^+-K^+ ATPase and Na^+-Ca^{2+} exchanger gene expression

The possibility that the observed changes in SL Na^+-K^+ ATPase and Na^+-Ca^{2+} exchange activities in the infarcted animals with or without IMP treatment may be occurring

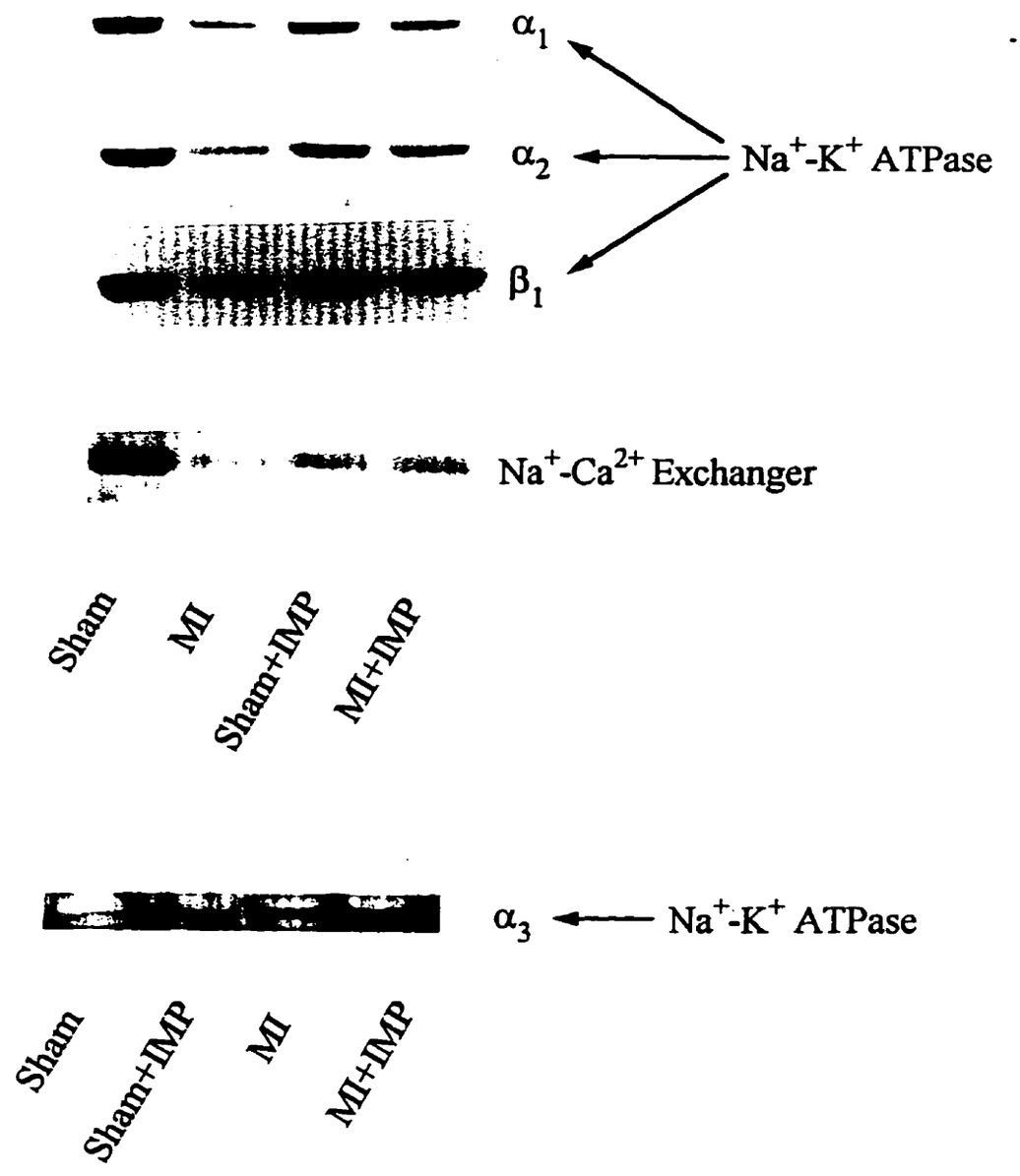

Figure 7 Typical Western blots for Na^+-K^+ ATPase and Na^+-Ca^{2+} exchanger in left ventricles from sham and 3 weeks infarcted (MI) rats with or without imidapril (IMP) treatment for 4 weeks. Immunoblots for different isoforms of Na^+-K^+ ATPase (α_1, α_2, α_3 and β_1) and Na^+-Ca^{2+} exchanger were obtained by using antibodies specific for each protein.

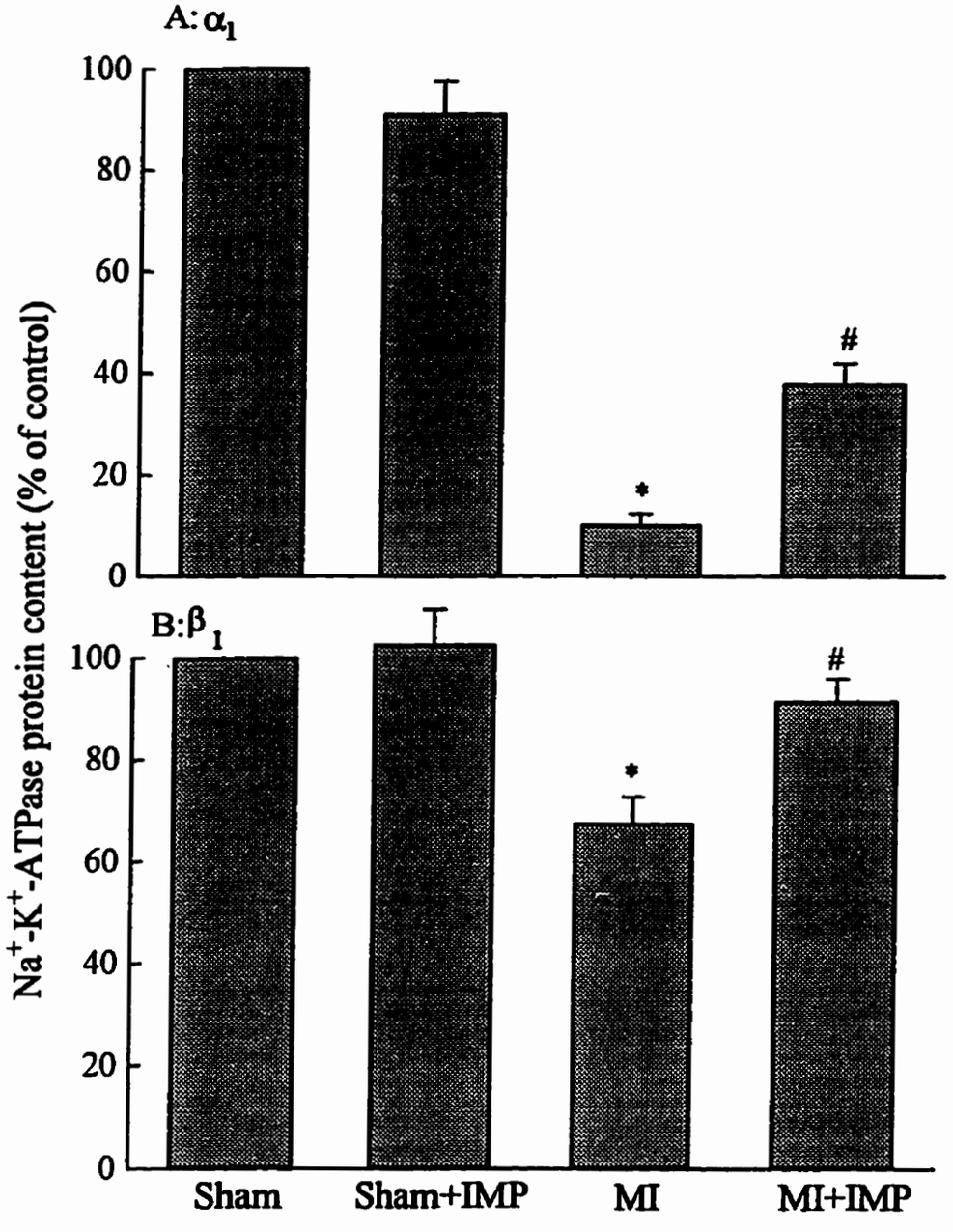

Figure 8 Protein content of α_1- and β_1-isoforms of Na^+-K^+ ATPase in left ventricles from sham and 3 weeks infarcted (MI) rats with or without imidapril (IMP) treatment for 4 weeks. IMP was given orally (1mg/kg, daily). Values are mean ± SE of 6 samples in each group. *$P < 0.05$ compared with sham control. #$P < 0.05$ compared with MI group.

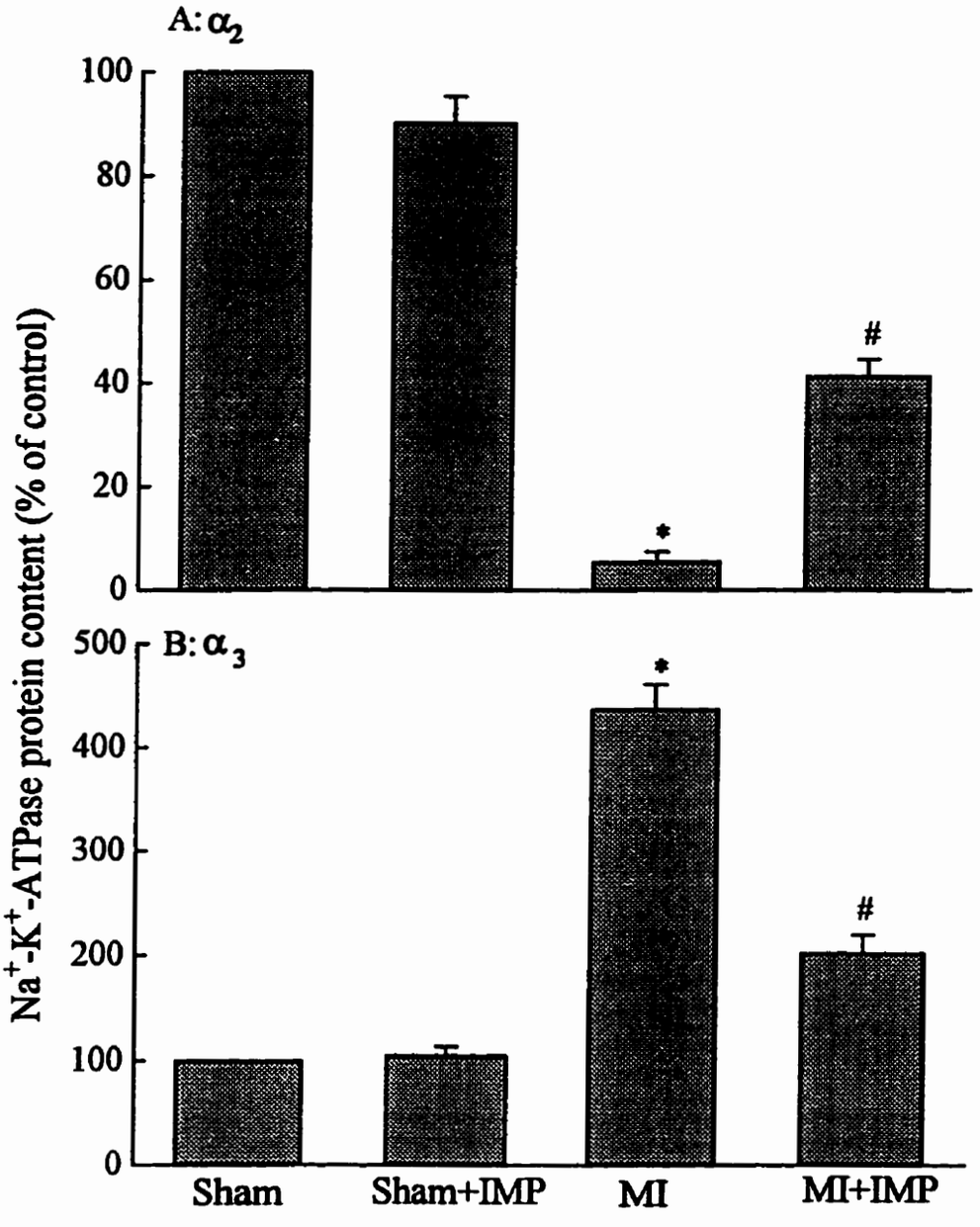

Figure 9 Protein content of α_2- and α_3-isoforms of Na^+-K^+ ATPase in left ventricles from sham and 3 weeks infarcted (MI) rats with or without imidapril (IMP) treatment for 4 weeks. IMP was given orally (1 mg/kg, daily). Values are mean ± SE of 6 samples in each group. *$P < 0.05$ compared with sham control. #$P < 0.05$ compared with MI group.

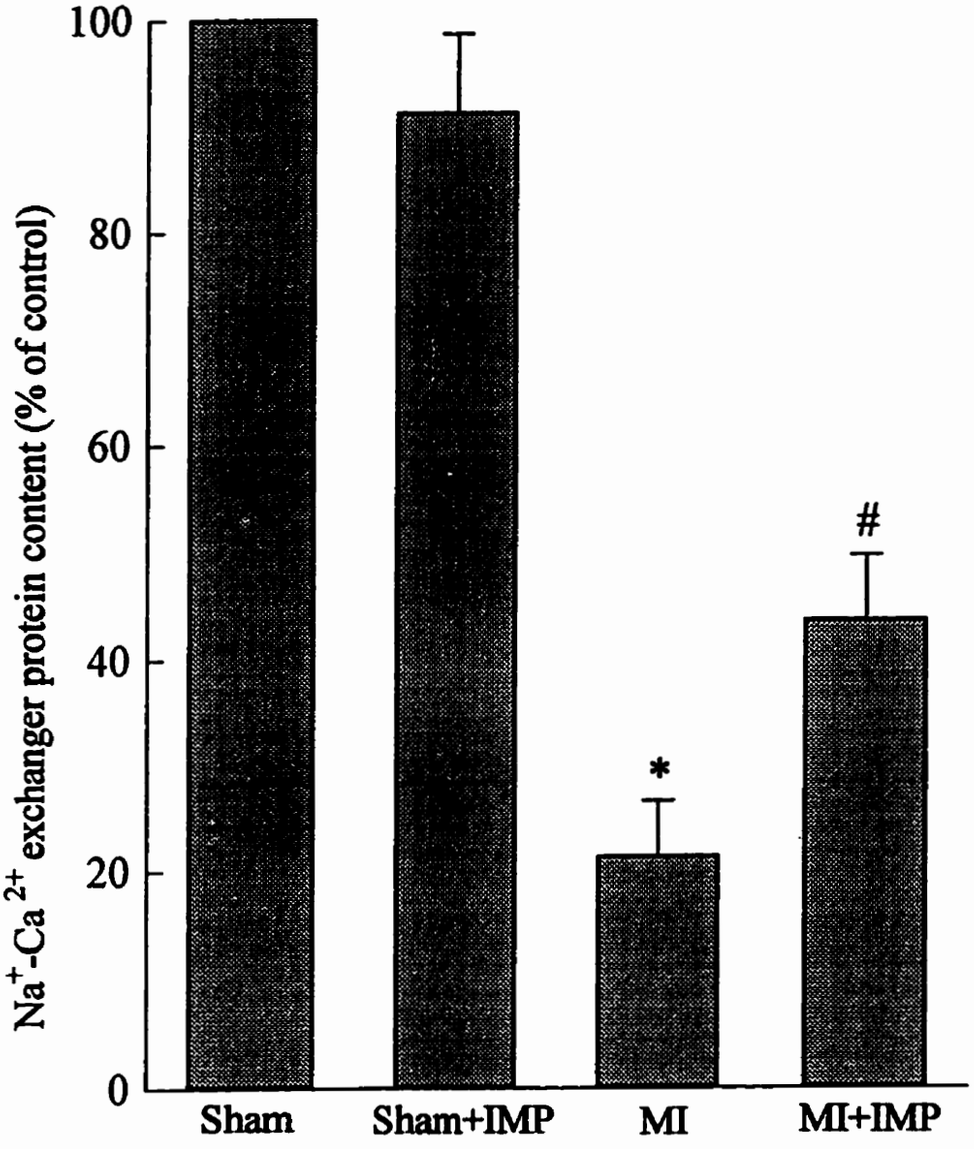

Figure 10 Protein content of Na^+-Ca^{2+} exchanger in left ventricles from sham and 3 weeks infarcted (MI) rats with or without imidapril (IMP) treatment for 4 weeks. IMP was given orally (1 mg/kg, daily). Values are mean ± SE of 6 samples in each group. *$P < 0.05$ compared with sham control. $^{\#}P < 0.05$ compared with MI group.

at the genetic level, we monitored gene expression for these proteins by measuring mRNA levels in Northern blots (Fig. 11). The quality of mRNA preparations from left ventricles of sham control and infarcted animals with or without IMP treatment was evident from 28S and 18S ribosomal RNA bands. mRNA levels of GAPDH and 18S were used as internal standards. Since mRNA levels for GAPDH and 18S in control preparations were not different from the experimental preparations, mRNA abundance for both Na^+-K^+ ATPase and Na^+-Ca^{2+} exchange proteins was normalized with GAPDH mRNA. It can also be seen from Fig. 11 that mRNA for four different isoforms of Na^+-K^+ ATPase (α_1, α_2, α_3 and β_1) were evident in the control and experimental preparation; the expression of α_3-isoform in the control heart was markedly less than the other isoforms. Densitometric analysis of the Northern blots indicate that the mRNA levels for α_1-, β_1- and α_2-isoforms were significantly depressed whereas that for α_3-isoform was markedly increased in the infarcted animals; these changes were attenuated by treatment of infarcted animals with IMP (Figs. 12 and 13). Similarly, a marked depression in mRNA level for Na^+-Ca^{2+} exchange in the infarcted animal was attenuated by treatment with IMP (Fig. 14). It can also be seen from Figs. 12, 13 and 14 that treatment of sham control animals with IMP did not exert any significant effect on the mRNA levels for different Na^+-K^+ ATPase isoforms as well as Na^+-Ca^{2+} exchanger protein.

6. SR Ca^{2+}-pump and Ca^{2+}-release channel activities and protein contents

The status of SR Ca^{2+}-pump in the failing heart was examined by studying the

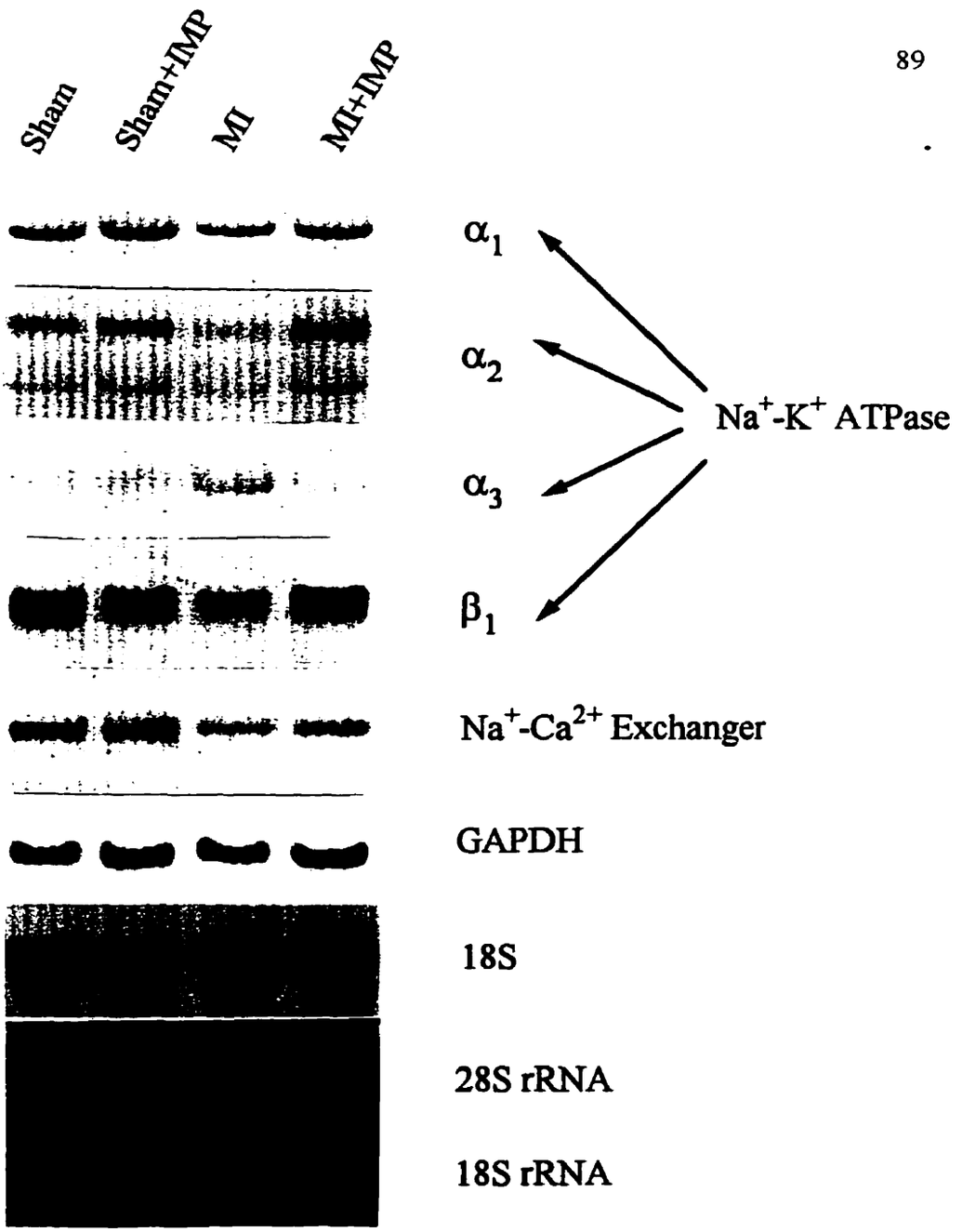

Figure 11 Typical Northern blots of Na^+-K^+ ATPase and Na^+-Ca^{2+} exchanger mRNA in left ventricles from sham and 3 weeks infarcted (MI) rats with or without imidapril treatment for 4 weeks. Blots for different isoforms of Na^+-K^+ ATPase (α_1, α_2, α_3 and β_1) were obtained whereas GADPH mRNA and 18S rRNA were used as internal standards for correcting loading variations in each sample. The quality of mRNA preparations is evident from 28S and 18S rRNA bands obtained by ethidium bromide staining of the rRNA agarose gel. IMP was given orally (1 mg/kg, daily).

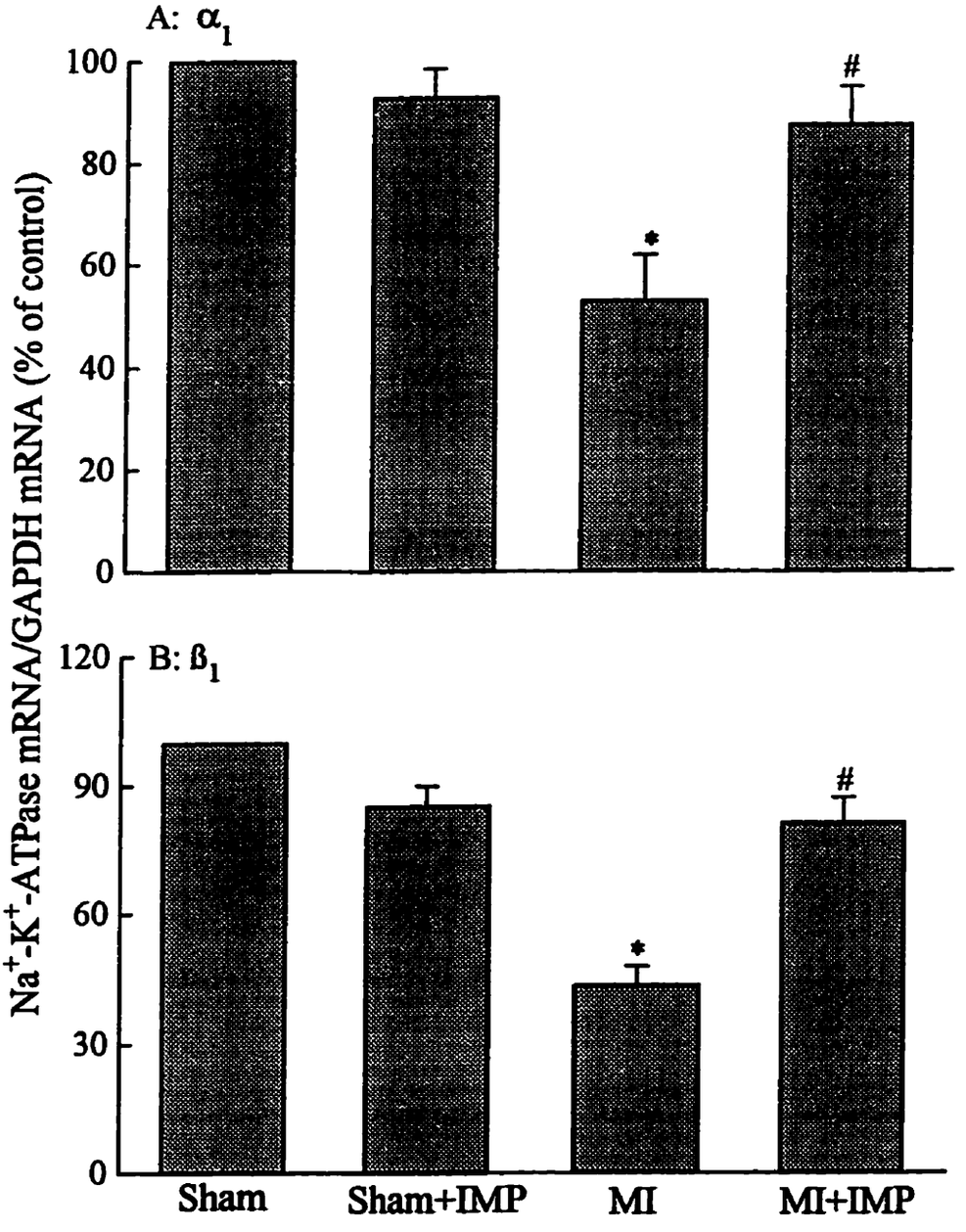

Figure 12 mRNA abundance of α_1- and β_1-isoforms of Na^+-K^+ ATPase in left ventricles from sham and 3 weeks infarcted (MI) rats with or without imidapril treatment for 4 weeks. IMP was given orally (1 mg/kg, daily). Values are mean ± SE of 6 samples in each group. *$P < 0.05$ compared with sham control. #$P < 0.05$ compared with MI group.

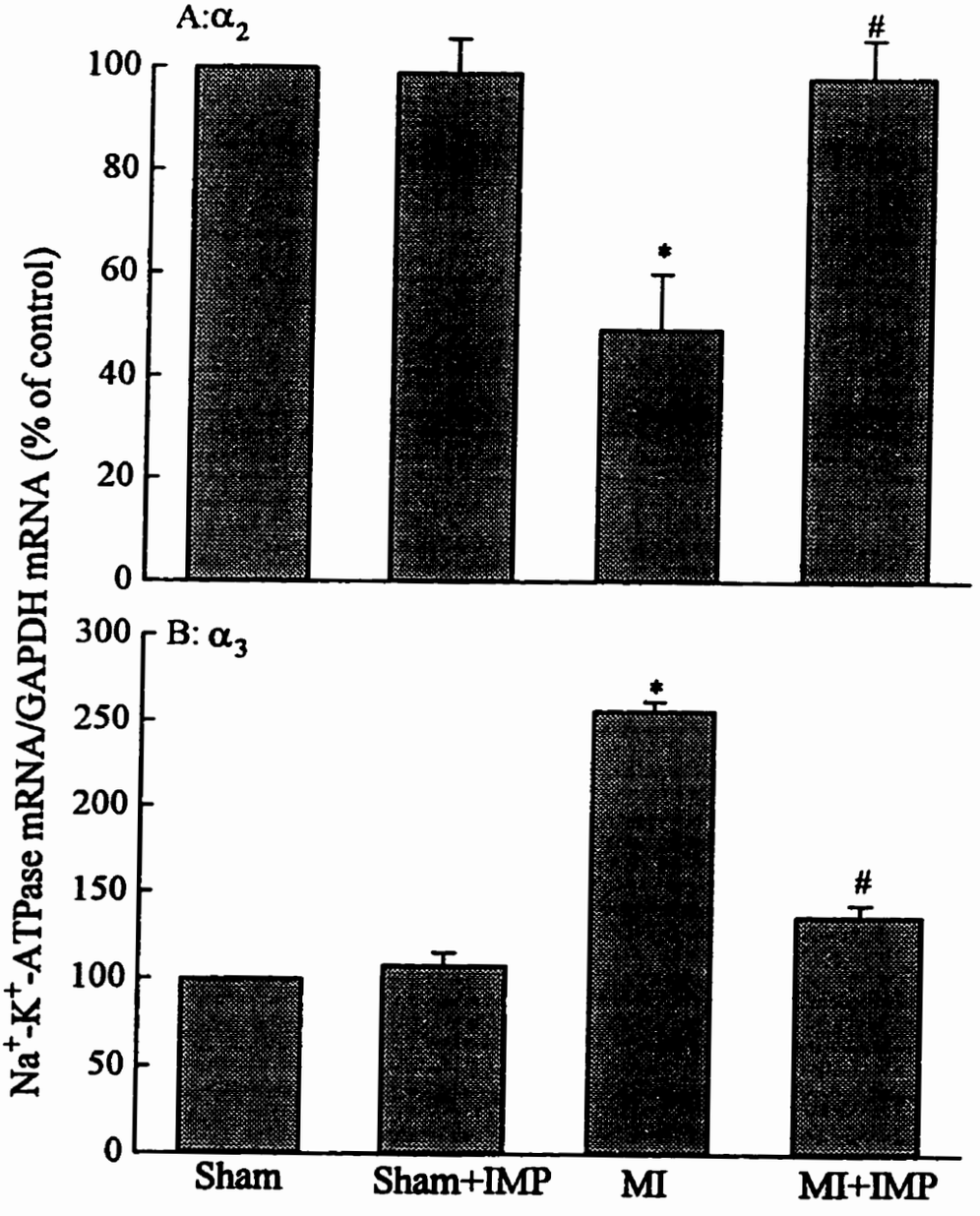

Figure 13 mRNA abundance of α_2- and α_3-isoforms of Na^+-K^+ ATPase in left ventricles from sham and 3 weeks infarcted (MI) rats with or without imidapril treatment for 4 weeks. IMP was given orally (1 mg/kg, daily). Values are mean ± SE of 6 samples in each group. *P < 0.05 compared with sham control. #P < 0.05 compared with MI group.

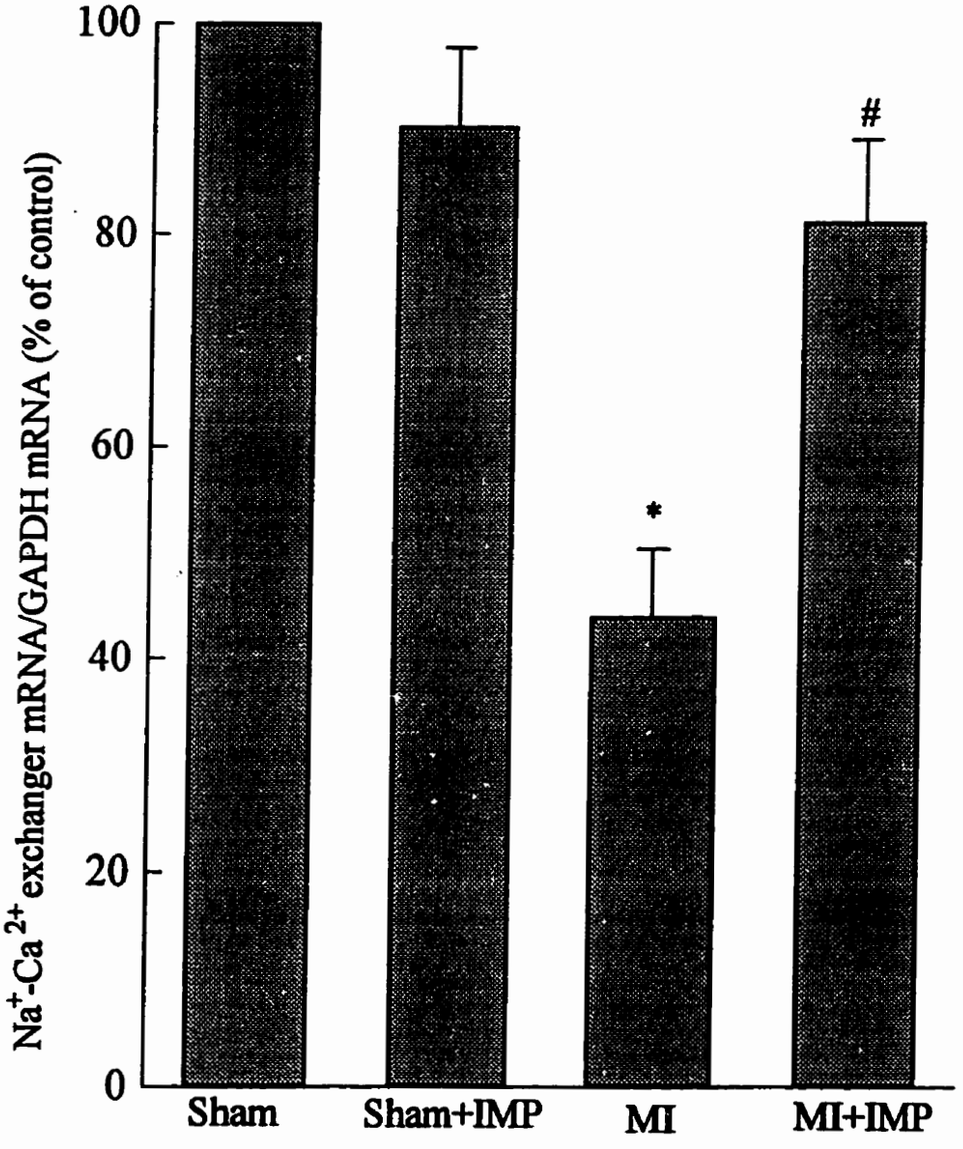

Figure 14 mRNA abundance of Na^+-Ca^{2+} exchanger in left ventricles from sham and 3 weeks infarcted (MI) rats with or without imidapril treatment for 4 weeks. IMP was given orally (1 mg/kg, daily). Values are means ± SE of 6 samples in each group. *$P < 0.05$ compared with sham control. $^{\#}P < 0.05$ compared with MI group.

ATP-dependent Ca^{2+}-uptake and Ca^{2+}-stimulated ATPase activities of the SR membrane fragments isolated from the left ventricles from sham control and infarcted animals with or without IMP treatment. Measurement of the Ca^{2+}-uptake activities at different times of incubation revealed that a depression in both the rate and capacity of SR to accumulate Ca^{2+} in the infarcted animals (Fig. 15). The ability of SR from failing hearts to accumulate Ca^{2+} was also depressed when measurements were made at different concentrations of Ca^{2+} in the incubation medium (Fig. 16). The depression in SR Ca^{2+}-uptake activities in the failing heart was partially prevented by the treatment of infarcted animals with IMP (Figs. 15 and 16). A marked decrease in Ca^{2+}-stimulated ATPase activity was evident in SR preparations from the failing heart; this depression was greatly prevented upon treating the infarcted animals with IMP (Fig. 17). Mg^{2+} ATPase activities in SR preparations from infarction animals with or without IMP treatment were not different significantly from the respective control values (Fig. 17). It can be seen from results shown in Figs. 15, 16 and 17 that treatment of sham control animals with IMP had no significant effect on the SR Ca^{2+}-uptake or Ca^{2+}-stimulated ATPase activities.

Alterations in Ca^{2+}-release channels in the SR preparations from infarcted animals with or without IMP treatment were monitored by measuring specific binding of ^{3}H-ryanodine, a well known antagonist of SR Ca^{2+}-release channels. The data in Fig. 18 show a depression ($P < 0.05$) in the specific binding of ^{3}H-ryanodine binding by the failing heart SR when measured at different concentrations of the antagonist. The Scatchard plot analysis of the data (Fig. 18) revealed a significant decrease in the B_{max} value for ^{3}H-

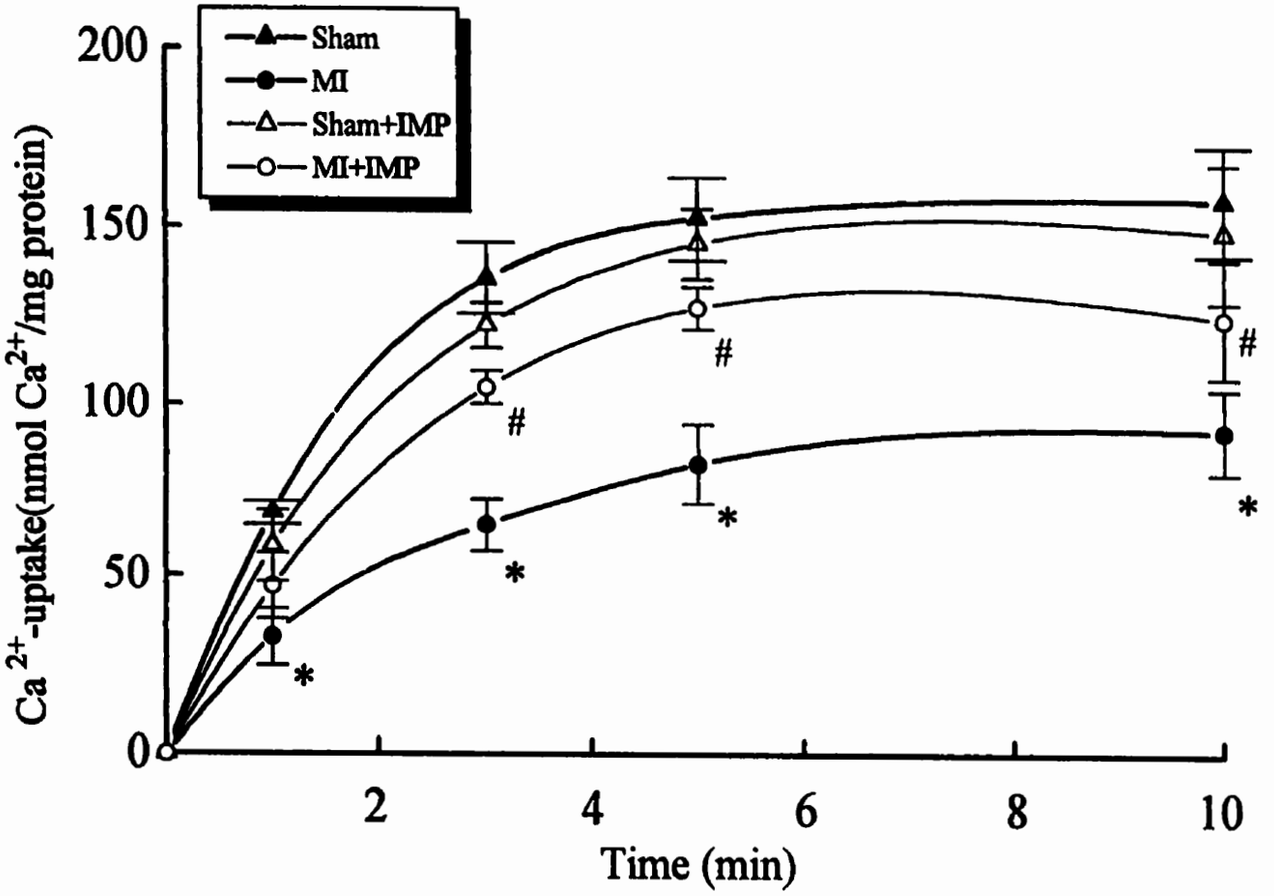

Figure 15 Left ventricular SR Ca^{2+}-uptake activity at different times of incubation in sham and 3 weeks infarcted rats with or without imidapril (IMP) treatment for 4 weeks. IMP (1 mg/kg, daily) was given orally before isolating SR from the viable left ventricle for the determination of Ca^{2+}-uptake activity. The concentration of Ca^{2+} in the incubation medium was 10 μM. Each value is a mean ± SE of 6 samples in each group. *P < 0.05 in comparison to the sham control. #P < 0.05 in comparison to the MI group.

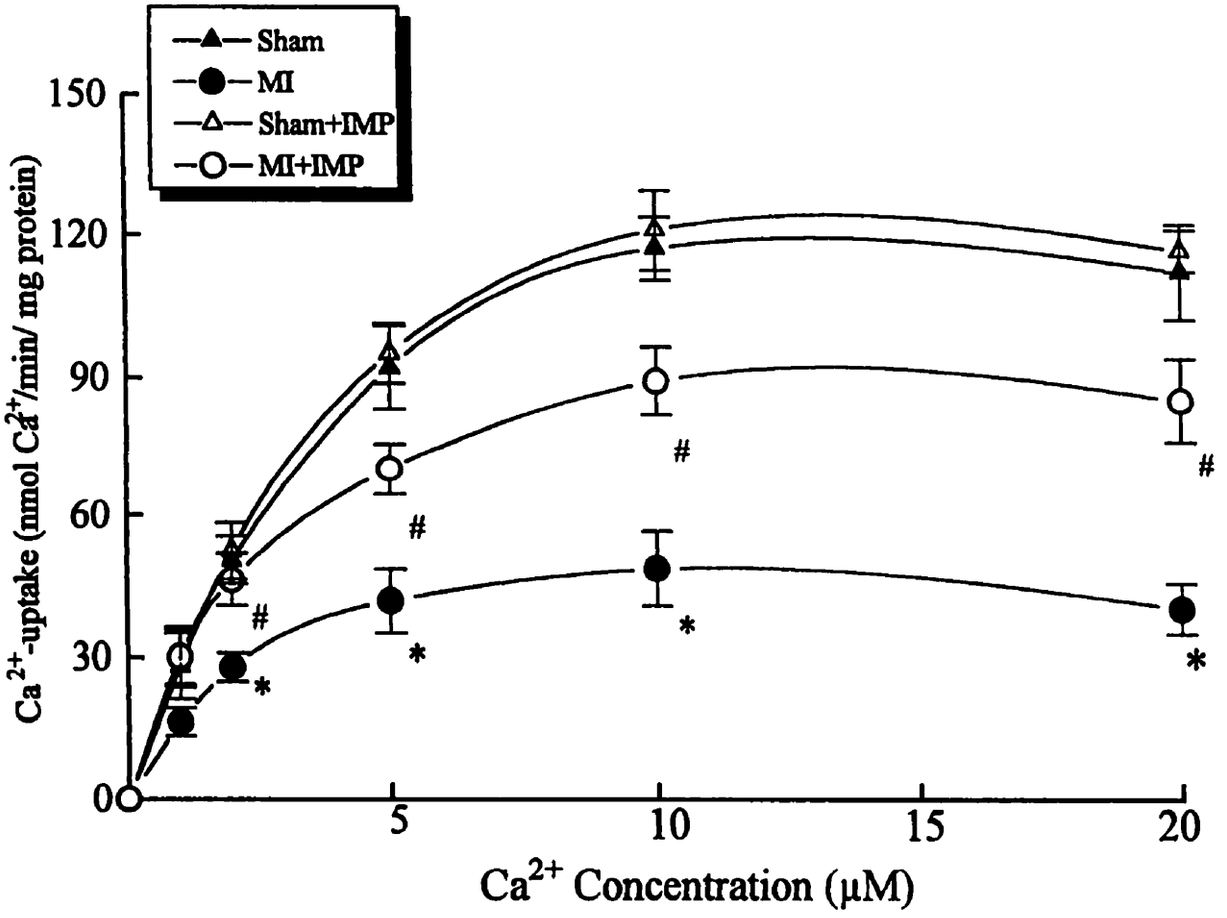

Figure 16 Left ventricular SR Ca^{2+}-uptake activity at different concentrations of Ca^{2+} in sham and 3 weeks infarcted (MI) rats with or without imidapril (IMP) treatment for 4 weeks. The time of incubation was 2 min. IMP was given orally (1 mg/kg, daily). Each value is a mean ± SE of 6 samples in each group. *P < 0.05 in comparison to the sham control. #P < 0.05 in comparison to the untreated MI group.

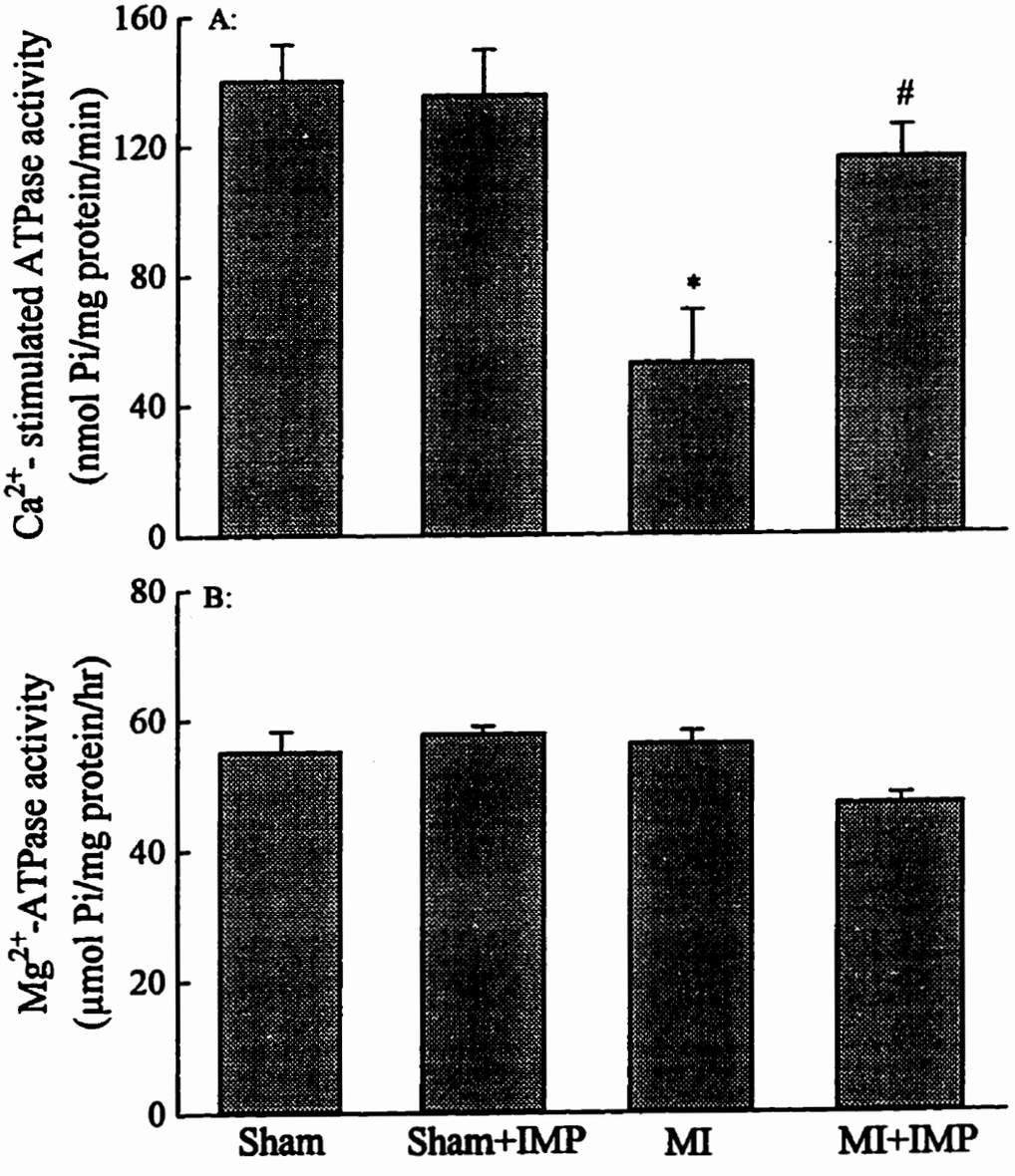

Figure 17 Left ventricular SR Ca^{2+}-stimulated ATPase and Mg^{2+} ATPase activities in sham and 3 weeks infarcted (MI) rats with or without imidapril (IMP) treatment for 4 weeks. IMP was given orally (1 mg/kg, daily). The concentration of Ca^{2+} for the Ca^{2+}-stimulated ATPase was 10 μM. Each value is a mean ± SE of 6 samples in each group. *$P < 0.05$ in comparison to the sham control. #$P < 0.05$ in comparison to the MI group.

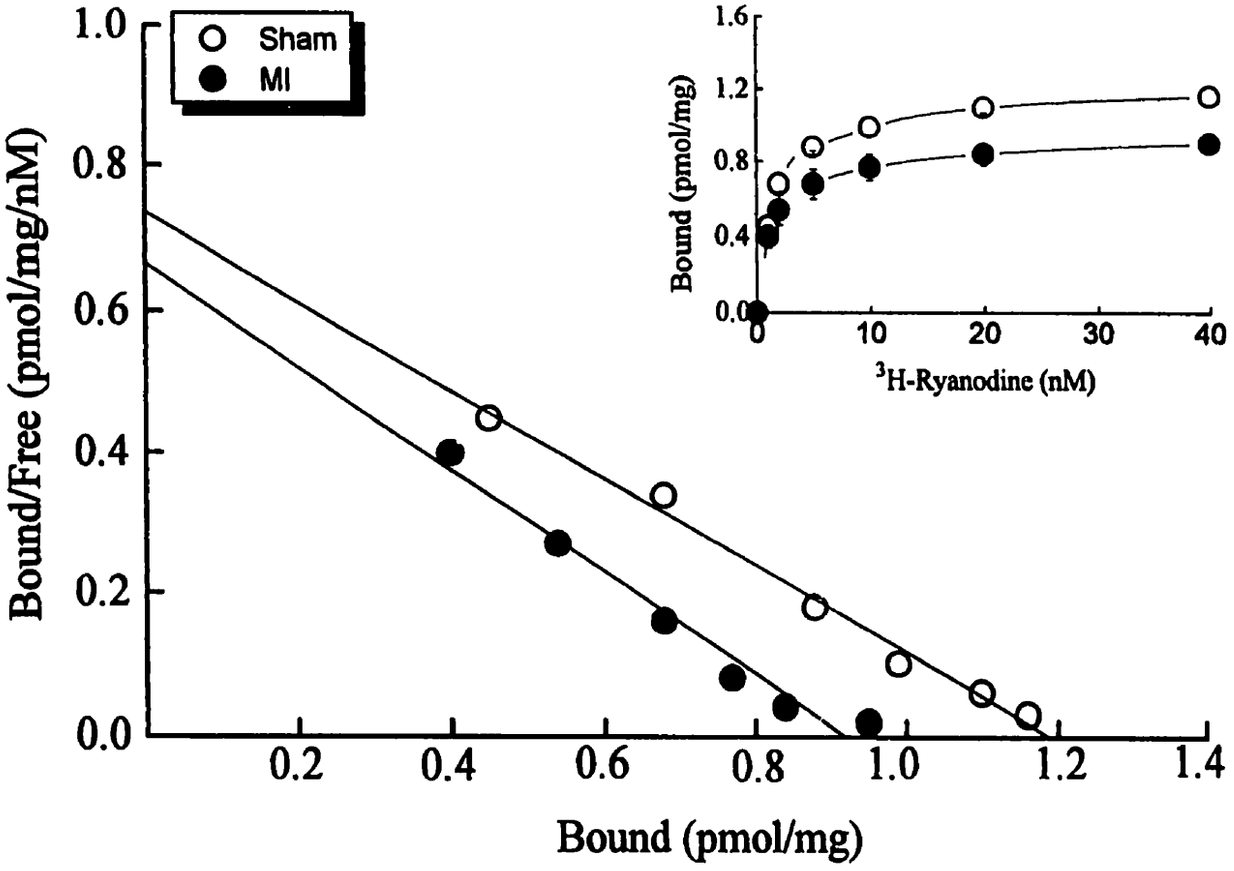

Figure 18 Scatchard plot of ^{3}H-ryanodine binding with SR membranes from left ventricles in sham and 3 weeks infarcted (MI) rats. Inset shows mean values ± SE of specific ^{3}H-ryanodine binding of 4 samples in each group.

ryanodine binding with the infarcted preparation without any changes in its affinity (Table 10). Treatment of infarcted animals with IMP completely prevented the depression in B_{max} value; this treatment did not exhibit any effect in the sham control animals (Table 10).

In order to show if the observed changes in the SR Ca^{2+}-pump and Ca^{2+}-release channels in the failing hearts were due to alterations in the contents of these proteins in SR membrane, Western blots of SR membranes (Fig. 19) were obtained by employing antibodies specific for these proteins. In addition, immunoblots for phospholamban, which is known to regulate the activity of Ca^{2+}-pump, were also obtained in SR membranes from sham control and infarcted animals with or without IMP treatment (Fig. 19). Densitometric analysis of the immunoblots revealed a depression in the relative protein contents for ryanodine receptor (SR Ca^{2+}-channel), Ca^{2+}-pump ATPase and phospholamban in the infarcted membranes in comparison to the sham control values. The depression in Ca^{2+}-channel and Ca^{2+}-pump protein contents was partially prevented whereas that in phospholamban protein content was fully prevented by treatment of infarcted animals with IMP (Fig. 20). Treatment of sham control animals with IMP had no effect on the Ca^{2+}-pump, Ca^{2+}-channel and phospholamban contents in the SR membrane (Fig. 20).

7. SR Ca^{2+}-pump and Ca^{2+}-release channel gene expression

The molecular mechanisms for the observed changes in SR Ca^{2+}-pump and Ca^{2+}-release channel activities in the failing hearts were investigated by monitoring mRNA levels specific for these proteins in the left ventricles. In addition, Northern blots for other

Table 10: B_{max} and K_d of ryanodine binding in the failing left ventricle of rats with or without imidapril treatment for 4 weeks starting at 3 weeks after coronary occlusion

	B_{max} (pmol/mg)	K_d (nM)
Sham	1.19 ± 0.05	1.58 ± 0.35
Sham + IMP	1.27 ± 0.06	1.66 ± 0.25
MI	0.90 ± 0.07*	1.25 ± 0.17
MI + IMP	1.24 ± 0.07#	1.27 ± 0.21

Values are means ± S.E. of 4 samples in each group and were calculated from the Scatchard plots. MI: myocardial infarction. IMP: imidapril (1 mg/kg, daily) was given orally. *$P < 0.05$ compared with sham control. #$P < 0.05$ compared with MI group.

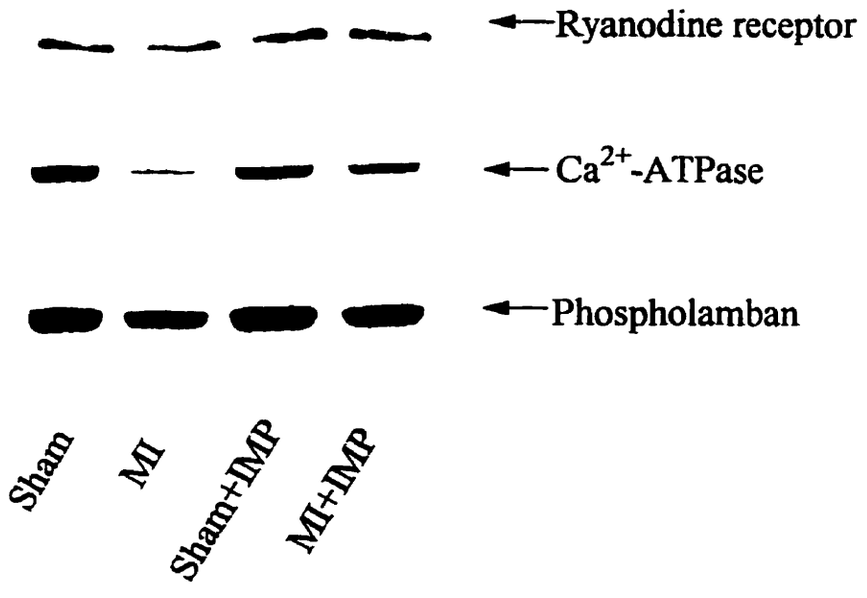

Figure 19 Typical Western blots of some SR proteins in left ventricles from sham and 3 weeks infarcted (MI) with or without imidapril (IMP) treatment for 4 weeks. Immunoblots obtained by using antibodies specific for each protein. IMP was given orally (1 mg/kg, daily).

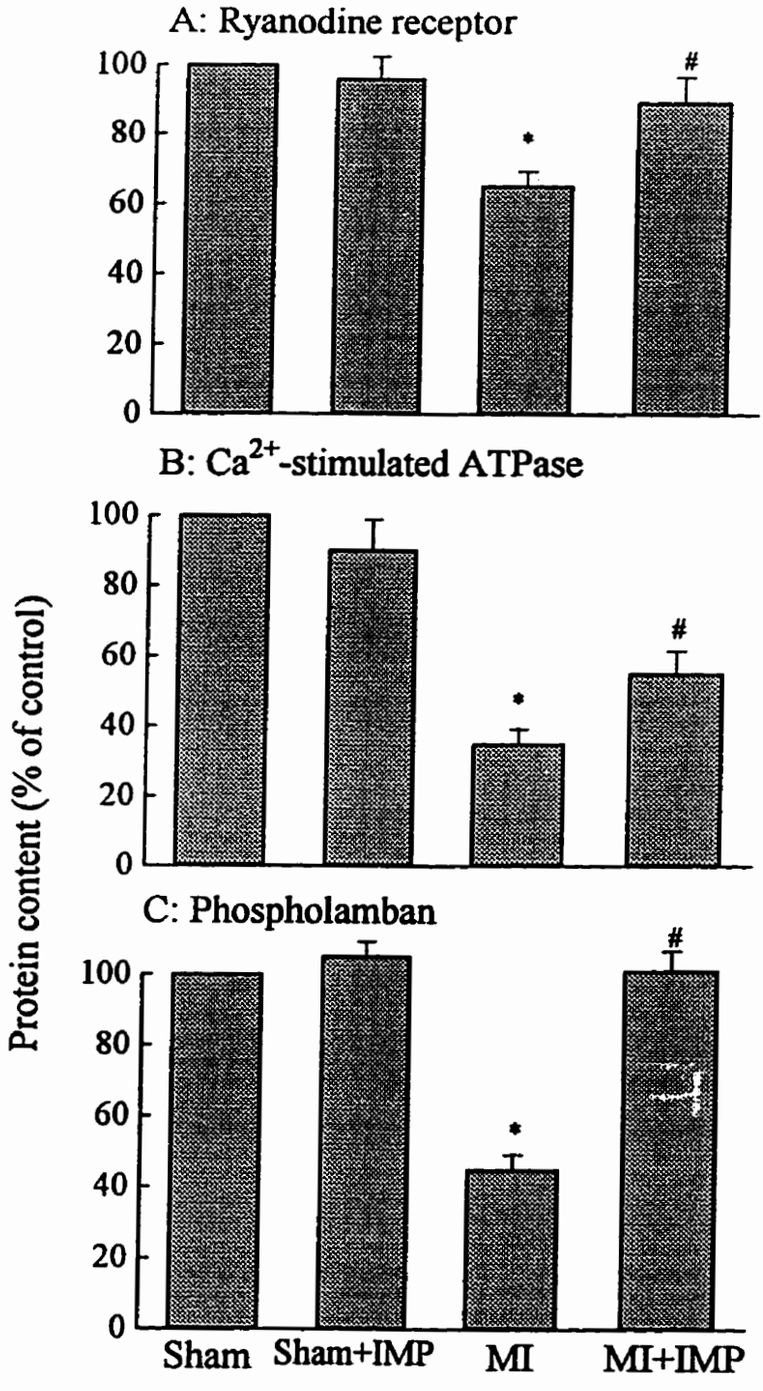

Figure 20 SR protein content of some SR proteins in left ventricles from sham and 3 weeks infarcted (MI) rats with or without imidapril (IMP) treatment for 4 weeks. IMP was given orally (1 mg/kg, daily). Values are mean ± SE of 6 samples in each group. *P < 0.05 compared with sham control. #P < 0.05 compared with MI group.

SR proteins, namely phospholamban, which regulates the SR Ca^{2+}-pump activity, and calsequestrin, which binds Ca^{2+} in the lumen of SR, were also obtained in the sham control and infarcted animals with or without IMP treatment (Fig. 21). Northern blots for GAPDH mRNA were used as an internal standard for normalization of the data. The quality of RNA preparations employed in these experiments is evident from the 28S and 18S bands (Fig. 21). Densitometric analysis of the Northern blots revealed a depression in the mRNA abundance for Ca^{2+}-pump, phospholamban and Ca^{2+}-release channel without any changes in the calsequestrin mRNA levels in the failing hearts (Figs. 22 and 23). The depression in Ca^{2+}-pump mRNA level was fully prevented whereas that in phospholamban or calsequestrin mRNA level was partially prevented by treatment of infarcted animals with IMP. The mRNA levels for SR Ca^{2+}-pump, Ca^{2+}-release channel, phospholamban and calsequestrin in the sham control animals were not affected by treatment with IMP (Figs. 22 and 23).

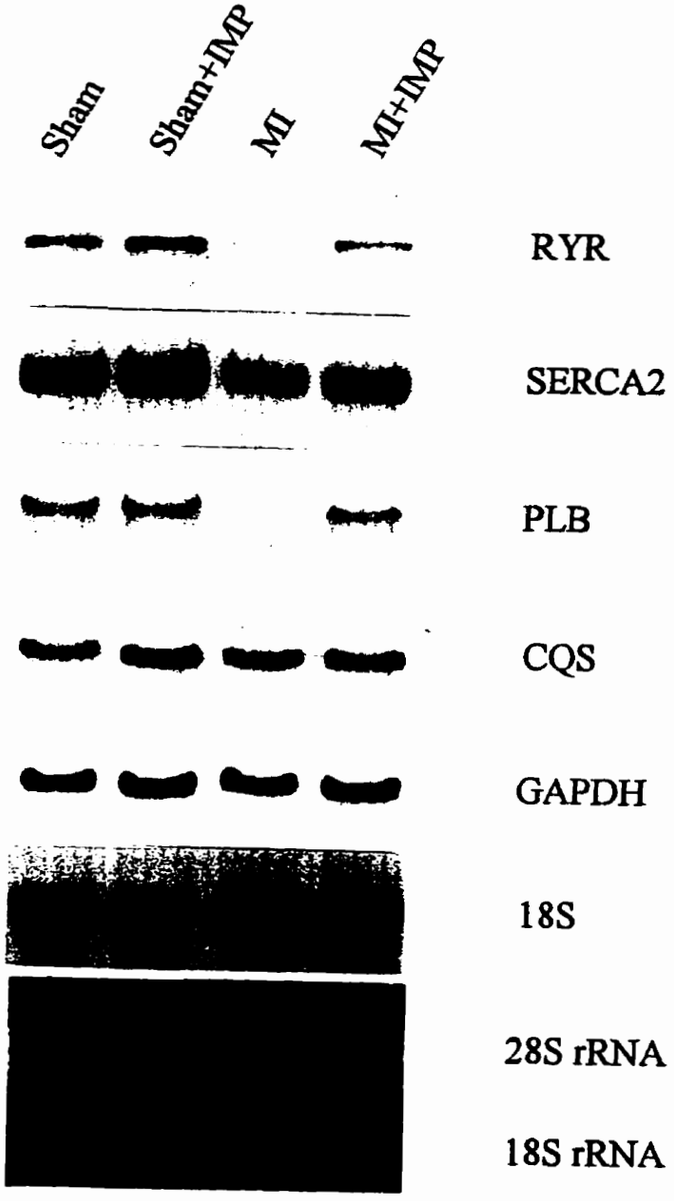

Figure 21 Typical Northern blots for some SR proteins in left ventricles from sham and 3 weeks infarcted (MI) rats with or without imidapril (IMP) treatment for 4 weeks. IMP was given orally (1 mg/kg, daily). Blots for ryanodine receptor (RYR), Ca^{2+}-stimulated ATPase (SERCA2), phospholamban (PLB) and calsequestrin (CQS) mRNA were obtained by using specific molecular probes. GAPDH mRNA level was used as internal standard for correcting loading variation in each group. The quality of mRNA preparation is apparent from the ethidium bromide staining of the 28S and 18S ribosomal RNA. Northern blots in lane 1: sham control; 2: sham + IMP; 3: MI, and 4: MI + IMP.

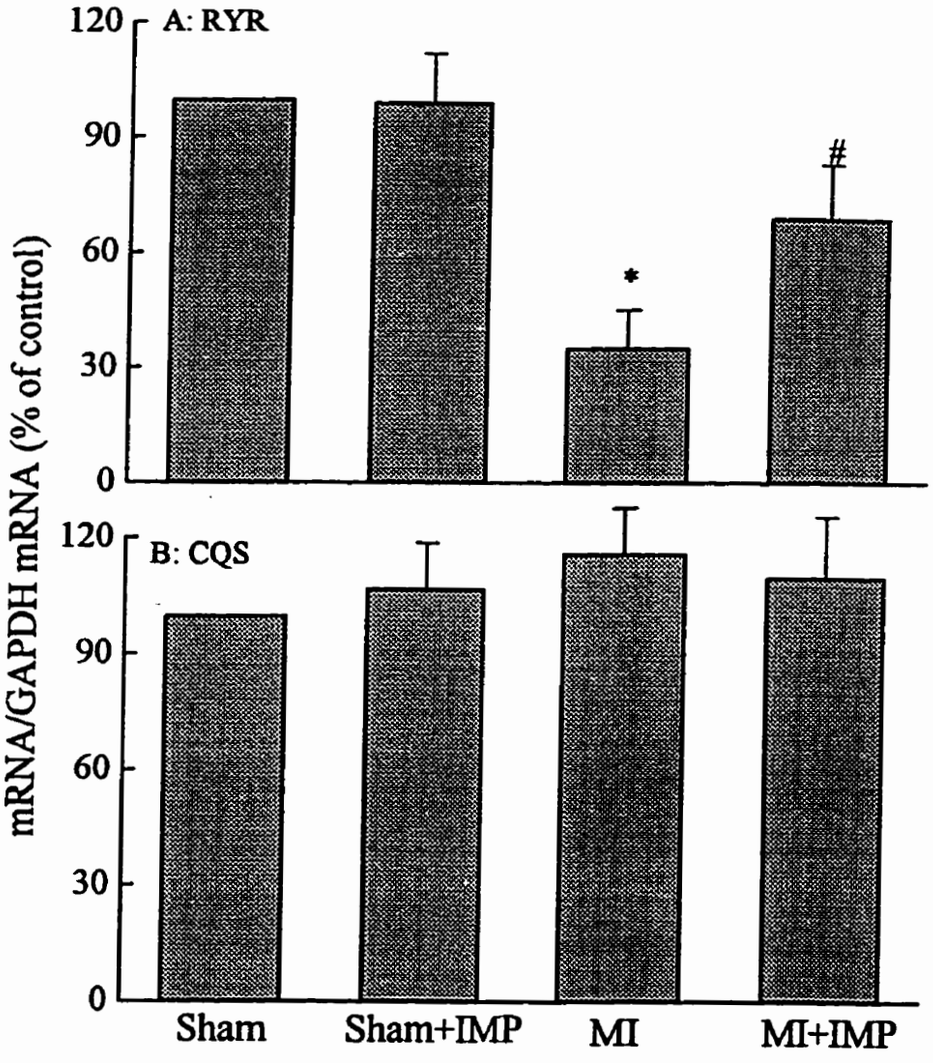

Figure 23 mRNA abundance for SR Ca^{2+}-release channel (RYR) and calsequestrin (CQS) in the left ventricle of sham and 3 weeks infarcted (MI) rats with or without imidapril (IMP) treatment for 4 weeks. The values are normalized with respect to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels and are expressed as % of sham control. Each value is a mean ± SE of 6 samples in each group. IMP was given orally (1 mg/kg, daily). *$P < 0.05$ in comparison to the sham control group. #$P < 0.05$ in comparison to the MI group.

which is released from the nerve terminals as were different cells (396-401), has been shown to influence many cellular functions. There is also an increasing evidence that ATP can serve as a potent extracellular signaling molecule and increase the cytosolic concentration of $[Ca^{2+}]_i$ in smooth and cardiac muscle cells (402-404). The sources of Ca^{2+} for the ATP-induced $[Ca^{2+}]_i$ have been suggested to be L-type Ca^{2+} channels in SL and Ca^{2+}-release as well as Ca^{2+}-uptake in SR. Therefore, both Ca^{2+} influx from extracellular compartment and Ca^{2+}-release from the intracellular stores are considered to account for the ATP-induced increase in $[Ca^{2+}]_i$. Our observation is consistent with a contribution of Ca^{2+} from both extracellular and intracellular Ca^{2+} store for the ATP induced rise in the $[Ca^{2+}]_i$ because verapamil and ryanodine were found to block this $[Ca^{2+}]_i$ increase. It should be noted that Ca^{2+} antagonist, verapamil, is a known inhibitor of L-type Ca^{2+} channels in sarcolemma whereas ryanodine is known to block the Ca^{2+}-induced Ca^{2+}-release channel in SR at high concentrations.

In the present study, the exogenous ATP significantly stimulated the $[Ca^{2+}]_i$ increase in myocytes from sham control. On the other hand, a marked reduction in this increase was observed in myocytes from the failing heart. In addition, the response to ATP was slower in myocytes of the infarcted group. Furthermore, our data showed not only a lower response to ATP but also to exogenous KCl in the failing left ventricular myocytes. These results indicated that heart failure due to myocardial infarction was associated with alterations at the level of sarcolemma and sarcoplasmic reticulum. This is consistent with previous reports from our laboratory indicating impairment of the cardiac membrane of sarcolemma and

sarcoplasmic reticulum in heart failure due to myocardial infarction (89, 90, 381, 388). This membrane damage is considered to serve as a mechanism of the ventricular pump failure showing lower rates of contraction (+dP/dt) and relaxation (-dP/dt) in rats with heart failure due to myocardial infarction (89, 90). Our results agree with the report by Cheung et al. (409) who showed significantly less cell shortening at high concentration of extracellular Ca^{2+}, and lower peak intracellular Ca^{2+} concentration in rats 3 weeks after coronary ligation. Likewise, the time required to reach from basal to peak $[Ca^{2+}]_i$ level and the rate of rise and decline of $[Ca^{2+}]_i$ were prolonged in the infarcted myocytes (409). In the same rat model, Capasso et al.(410) showed that the peak systolic Ca^{2+} was depressed by 22% and the time to peak Ca^{2+} was prolonged. Moreover, the time for Ca^{2+} to return to diastolic levels was also prolonged in the failing left ventricular myocytes. Since no difference in K_d and B_{max} of ATP receptors was seen in any of the groups, it is evident that imidapril may not have improved the response to exogenous ATP in failing myocytes through ATP receptors. On the other hand, imidapril prevented the impairment of cardiomyocyte responses to ATP by improving the cardiac SL and SR function and subsequent Ca^{2+} handling in the failing hearts. Nonetheless, the depressed response of the isolated hearts as well as cardiomyocytes from the infarcted animals can be taken to suggest that there occurs a loss of purinergic induced signal transduction mechanism in heart failure.

3. Effect of imidapril on SL Na^+-K^+ ATPase and Na^+-Ca^{2+} exchanger

Another major finding of this study was with respect to the status of SL Na^+-K^+

were also decreased in the failing heart. This suggested that a reduction in the gene expression and protein content of α_1-, α_2- and β_1-isoforms of Na^+-K^+ ATPase may contribute to the loss of the activity of Na^+-K^+-ATPase in heart failure due to myocardial infarction. Such a conclusion does not exclude the possibility that increased gene expression and protein content of α_3-isoform of Na^+-K^+ ATPase may play an important role in the changes reported here.

SL Na^+-Ca^{2+} exchanger has been suggested to participate in the efflux of Ca^{2+} from the cytosolic compartment of the cardiomyocytes. Although the importance in Na^+-Ca^{2+} exchanger in cardiac excitation-contraction coupling has been demonstrated, the status of the Na^+-Ca^{2+} exchanger activity in heart failure remains controversial. Studer et al. (111) found that cardiac Na^+-Ca^{2+} exchanger was increased to about 55% and 41% in cardiomyopathy and coronary artery disease in patients. Increased Na^+-Ca^{2+} exchanger mRNA expression has also been reported in other types of heart failure (414). The results of our present study confirmed an earlier report from our laboratory (89) which demonstrated a significant depression in Na^+-Ca^{2+} exchange in SL vesicles isolated from left ventricles of myocardial infarction. Our present study demonstrated that not only a decrease in the Na^+-dependent Ca^{2+}-uptake was evident, the Na^+-Ca^{2+} exchanger mRNA level and protein content were also depressed in heart failure following myocardial infarction. The gene expression and protein content changes for Na^+-Ca^{2+} exchanger may be considered as the basis of the loss in Na^+-Ca^{2+} exchanger activity in the failing heart. It is possible that the regulation of Na^+-Ca^{2+} exchanger may be different in various pathological conditions because Zhang et al. (415)

have also demonstrated a lower Na^+-Ca^{2+} exchanger current in the postinfarcted myocytes.

We have demonstrated that imidapril significantly improved the activity of Na^+-K^+ ATPase and Na^+-dependent Ca^{2+}-uptake as well as their mRNA expression and protein levels. These observations are consistent with other reports showing the beneficial effects of some inhibitors on Na^+-K^+ ATPase. In this regard, Howl et al. (416) indicated that captopril increased the Na^+-K^+ pump proteins in cardiac myocytes; similar results were obtained by using another ACE inhibitor, enalapril, but not by a vasodilator, hydrazine. A recent report by Ottlecz (417) showing that captopril significantly ameliorated the depression in retina Na^+-K^+ ATPase in streptozotocin-induced diabetic rats has also appeared in the literature. These data appear to suggest that the beneficial effects of imidapril on Na^+-K^+ ATPase and Na^+-dependent Ca^{2+}-uptake activities in the failing heart due to myocardial infarction may be a consequence of improvement through the Na^+-K^+ ATPase and Na^+-Ca^{2+} exchanger gene expression. These observations can also be interpreted to suggest that there occurs a remodeling of the SL membrane, which is associated with alterations in the Na^+-K^+ ATPase and Na^+-Ca^{2+} exchange activities. Accordingly, imidapril treatment may be exerting beneficial effects on the failing hearts by preventing the remodeling of the SL membrane.

4. Effect of imidapril on SR Ca^{2+}-transport and gene expression

Intracellular Ca^{2+} homeostasis in cardiomyocytes is maintained mainly by Ca^{2+}-release from and Ca^{2+}-uptake by SR. The SR Ca^{2+}-stimulated ATPase which mediates the uptake of Ca^{2+} into the SR, and the ryanodine receptor which mediates the release of Ca^{2+} from SR,

are known to play an important role in cardiac relaxation and contraction, respectively (2). Depression of the cardiac pump function, a hallmark of heart failure, has been associated with a reduction in cardiac SR Ca^{2+}-transport (2, 112-115). The results in the present study confirmed previous reports from our laboratory that reduced cardiac function in failing hearts due to myocardial infarction was associated with a reduction of the Ca^{2+}-stimulated ATPase and Ca^{2+}-uptake activities (90, 392).

A decrease in the SR Ca^{2+} transport gene expression has been reported in different pathological conditions. de la Bastie et al. (96) first indicated the decreased SR Ca^{2+} ATPase mRNA expression in severe cardiac hypertrophy induced by pressure overload. Furthermore, changes in mRNA levels for SR Ca^{2+}-pump protein were demonstrated in failing hearts of experimental animals and humans (99); these conditions included cardiomyopathy, coronary artery disease, primary pulmonary hypertension and congenital heart disease. Recently a depression in SR Ca^{2+} ATPase mRNA in the rat model of heart failure has also been shown (104); the results indicated that the level of SR Ca^{2+} ATPase mRNA was positively correlated with cardiac functional indexes and SR functions (89, 90). In the present study, we showed that the mRNA levels for SR ryanodine receptor, Ca^{2+}-pump ATPase and phospholamban were altered in parallel with changes in cardiac function in failing heart following myocardial infarction. A wide variety of changes in protein levels of SR Ca^{2+}-pump ATPase, phospholamban and ryanodine receptor have been reported in different pathological conditions (95, 117, 118). Decreased SR Ca^{2+}-pump ATPase and phospholamban protein levels have been shown in human dilated cardiomyopathy and

ischemic heart failure (115). The protein levels of SR Ca^{2+}-pump ATPase and phospholamban were 85% and 68% of control in aorta banded guinea pigs (95). Hasenfuss et al. (418) reported the reduced protein level of SR Ca^{2+}-pump ATPase by 36% and this decrease was closely related to SR Ca^{2+}-uptake decrease. Meyer et al. (117) showed that SR Ca^{2+}-uptake and the affinity of SR Ca^{2+}-pump ATPase for Ca^{2+} were significantly depressed while no change in the protein level of phospholamban and ryanodine receptor were found. Furthermore, a significant reduction in the SR Ca^{2+}-pump ATPase mRNA was associated with no change in the protein contents of phospholamban and ryanodine receptor in the failing heart (118).

The Ca^{2+}-pump ATPase (SERCA2) is regulated by another SR protein, phospholamban, a small transmembrane homopentamer of 52 amino acids, that is co-localized with SERCA2 in the longitudinal SR membrane. In its unphosphorylated form, phospholamban is known to inhibit the Ca^{2+}-pump ATPase activity by decreasing its affinity for Ca^{2+}. When phosphorylated, phospholamban is released from its binding site and the apparent sensitivity for Ca^{2+} is markedly increased. Our results show a parallel decrease in phospholamban mRNA and SERCA2 mRNA with depressed protein levels in myocardial infarcted rats indicating abnormalities in the regulation of SR Ca^{2+}-handling in the failing heart. There is another important mechanism in SR which regulates Ca^{2+} mobilization in the heart and is known as Ca^{2+}-induced Ca^{2+}-release channel (ryanodine receptor). In our experiment, the SR mRNA expression for both Ca^{2+}-uptake and -release proteins were decreased. Such a defect in both SR Ca^{2+} release and Ca^{2+}-uptake mechanisms can be seen

to result in the systolic and diastolic function in the failing heart. The level of calsequestrin mRNA did not change significantly in heart failure caused by coronary artery ligation showing that the alterations in the expression of SR proteins in the failing heart is somewhat specific in nature.

Imidapril treatment for 4 weeks showed improvement in the SR Ca^{2+}-uptake, mRNA abundance and protein levels of ryanodine receptor, Ca^{2+}-pump ATPase, and phospholamban. Since imidapril treatment also resulted in improvement of cardiac function, the beneficial effect of imidapril may be due to its effect on SR function through its influence on the SR Ca^{2+}-pump ATPase, phospholamban and ryanodine receptor gene expression and corresponding protein levels. To our knowledge, this is the first report regarding the beneficial effect of ACE inhibitors on SR gene expression and protein content in heart failure due to myocardial infarction. A recent study indicated that benazepril, an ACE inhibitor, significantly normalized the reduced SR Ca^{2+} ATPase gene expression in renal hypertensive rats. Taken together, we may conclude that imidapril may improve cardiac function in heart failure due to myocardial infarction through a prevention of the SR membrane remodeling by affecting the SR Ca^{2+}-transport gene expression and therefore increasing the SR Ca^{2+}-transport function in heart failure.

VII. REFERENCES

1. Ringer SA. A further contribution regarding the influence of different constituents of the blood on the contraction of the heart. J Physiol (Lond) 4:29-42, 1883.
2. Dhalla NS, Pierce GN, Panagia V, Singal PK, Beamish RE. Calcium movements in relation to heart function. Basic Res Cardiol 77:117-139, 1982.
3. Carafoli E. Membrane transport of calcium: An overview. Methods Enzymol 157:3-11, 1988.
4. Bers DM, Bassani JW, Bassani RA. Competition and redistribution among calcium transport system in rabbit cardiac myocytes. Cardiovasc Res 27:1772-1777, 1993.
5. Negretti N, O'Neill SC, Eisner DA. The relative contributions of different intracellular and sarcolemmal systems to relaxation in rat ventricular myocytes. Cardiovasc Res 27:1826-1830, 1993.
6. Reuter H, Seitz N. The dependence of calcium efflux from cardiac muscle on temperature and external ion composition. J Physiol (Lond) 195:451-470, 1968.
7. Reeves JP, Hale CC. The stoichiometry of the cardiac sodium-calcium exchange system. J Biol Chem 259:7733-7739, 1984.
8. Philipson KD, Longoni S, Ward R. Purification of the cardiac Na^+-Ca^{2+} exchange protein. Biochim Biophys Acta 945:298-306, 1988.
9. Nicoll DA, Longoni S, Philipson KD. Molecular cloning and functional expression of the cardiac sarcolemmal Na^+-Ca^{2+} exchanger. Science 250:562-565, 1990.
10. Kofuji P, Hadley RW, Kieval RS, Leserer WL, Schulze DH. Expression of the Na-

Ca exchanger in diverse tissues: a study using the cloned human cardiac Na-Ca exchange. Am J Physiol 263:C1241-C1249, 1992.

11. Low W, Kasir J, Rahamimoff H. Cloning of the rat heart Na^+-Ca^{2+} exchanger and its functional expression in HeLa cells. FEBS Lett 316:63-67, 1993.

12. Komuro I, Wenninger E, Philipson KD, Izumo S. Molecular cloning and characterization of the human cardiac Na/Ca exchanger cDNA. Proc Natl Acad Sci USA 89:4769-4773, 1992.

13. Hilgemann DW, Nicoll DA, Philipson KD. Charge movement during Na^+ translocation by native and cloned cardiac Na^+/Ca^{2+} exchanger. Nature 352:715-718, 1991.

14. Niggli E, Lederer WJ. Molecular operations of the sodium-calcium exchanger revealed by confirmation currents. Nature 349:621-624, 1991.

15. Frank JS, Mottino G, Reid D, Molday RS, Philipson KD. Distribution of the Na^+-Ca^{2+} exchange protein in mammalian cardiac myocytes: An immunofluorescence and immunocolloidal Gold-labelling study. J Cell Biol 117:337-345, 1992.

16. DiPolo R. Calcium influx in internally dialyzed squid giant axons. J Gen Physiol 73:91-113, 1979.

17. Philipson KD. The cardiac Na^+-Ca^{2+} exchanger. In: *Calcium and the Heart.* Glenn AL (ed). New York: Raven Press Ltd., 1990, pp. 85-108.

18. Hilgemann DW. Regulation and deregulation of cardiac Na^+-Ca^{2+} exchange in giant excised sarcolemmal membrane patches. Nature 344:242-245, 1990.

19. Matsuoka S, Nicoll DA, Hryshko LV, Levitssky DO, Weiss JN, Philispon KD. Regulation of the cardiac Na^+-Ca^{2+} exchange by Ca^{2+}. Mutational analysis of the Ca^{2+}-binding domain. J Gen Physiol 105:403-420, 1995.

20. Schulze D, Kofuji P, Hadley R, Kirby MS, Kieval RS, Doering A, Niggli E, Lederer WJ. Sodium/calcium exchanger in heart muscle, molecular biology, cellular function, and its special role in excitation-contraction coupling. Cardiovasc Res 27:1726-1734, 1993.

21. Shigekawa M, Iwamoto T, Wakabayashi S. Phosphorylation and modulation of Na/Ca exchanger in vascular smooth muscle cells. Ann NY Acad Sci 779:249-257, 1996.

22. Lin L, Kao L, Westhead EW. Agents that promote protein phosphorylation increase catecholamine secretion and inhibit the activity of the Na/Ca exchanger in bovine chromaffin cells. Ann NY Acad Sci 779:395-396, 1996.

23. Philipson KD, Nicoll DA, Matsuoka S, Hryshko LV, Levitsky DO, Weiss JN. Molecular regulation of the Na^+-Ca^{2+} exchanger. Ann NY Acad Sci 779:20-28, 1996.

24. Bean BP. Classes of calcium channel in vertebrate cells. Ann Rev Physiol 51:367-384, 1989.

25. Gibbons WR, Fozzard HA. Relationships between voltage and tension in sheep cardiac Purkinje fibers. J Gen Physiol 65:345-356, 1975.

26. Nilius B. A novel type of cardiac calcium channel in ventricular cells. Nature 316:443-446, 1985.

27. Hagiwara N, Irisawa H, Kameyama M. Contribution of two types of calcium currents to the pacemaker potentials of rabbit sino-atrial node cells. J Physiol (Lond) 395:233-253, 1988.

28. Bogdanov KY, Ziman BD, Spurgeon HA, Lakatta EG. L- and T-type calcium currents differ in finch and rat ventricular cardiomyocytes. J Mol Cell Cardiol 27:2581-2593, 1995.

29. Balke CW, Gold MR. Calcium channels in the heart: An overview. Heart Disease and Stroke 1:398-403, 1992.

30. Catterall WA. Structure and function of voltage-sensitive ion channel. Science 242:50-61, 1988.

31. Singer-Lahat D, Biel M, Lotan I, Flockerzi V, Hofmann F, Dascal N. The roles of the subunits in the function of the calcium channel. Science 253:1553-1557, 1991.

32. Catterall WA. Functional subunit structure of voltage-gated calcium channels. Science 253:1499-1500, 1991.

33. Reuter H. Calcium channel modulation by neurotransmitters, enzymes and drugs. Nature 301:569-574, 1983.

34. Maki T, Gruver EJ, Davidoff AJ, Izzo N, Toupin D, Colucci W, Marks AR. Regulation of calcium channel expression in neonatal myocytes by catecholamines. J Clin Invest 97:656-663, 1996.

35. Sumii K, Sperelakis N. cGMP-dependent protein kinase regulation of the L-type Ca^{2+} current in rat ventricular myocytes. Circ Res 77:803-812, 1995.

36. Sperelakis N. Properties of calcium channels in cardiac muscle and vascular smooth muscle. Mol Cell Biochem 99:97-109, 1990.

37. Katz AM. Calcium channel diversity in the cardiovascular system. J Am Coll Cardiol 28:522-529, 1996.

38. Dunham ET, Glynn LM. Adenosine triphosphatase activity and the active movements of alkali metal ions. J Physiol (Lond) 156:274-293, 1961.

39. Schatzmann HJ. The calcium pump of the surface membrane and the sarcoplasmic reticulum. Annu Rev Physiol 51:473-485, 1989.

40. Monteith GR, Roufogalis BD. The plasma membrane calcium pump — a physiological perspective on its regulation. Cell Calcium 18:459-470, 1995.

41. Carafoli E. The plasma membrane calcium pump: Structure, function, regulation. Biochim Biophys Acta 1101:266-267, 1992.

42. Suju M, Davila M, Poleo G, Docampo R, Benaim G. Phosphatidylethanol stimulates the plasma-membrane calcium pump from human erythrocytes. Biochem J 317:933-938, 1996.

43. Carafoli E. How calcium crosses plasma membranes including the sarcolemma. In: *Calcium Antagonists and Cardiovascular Disease*. Opie L (ed). New York: Raven Press Ltd., 1994, pp 29-41.

44. Skou JC. The energy coupled exchange of Na^+ for K^+ across the cell membrane. The Na^+, K^+-pump. FEBS Lett 268:314-324, 1990.

45. Sulakhe PV, Elimban V, Dhalla NS. Characterization of a partially purified Na^+, K^+-

ATPase from dog heart. Advan Myocardial Dhalla NS (ed.) 1985, pp. 249-257.

46. von Schatzmann HJ. Herzglykoside als hemmstoffe für den aktiven kalium — und natriumtransport durch die erythrocytenmembran. Helv Physiol Pharmacol Acta 11:346-354, 1953.

47. Lingrel JB. Na,K-ATPase: Isoform structure, function and expression. J Bioener Biomem 24:263-270, 1992.

48. Sweadner KJ. Isozymes of Na,K-ATPase. Biochem Biophys Acta 988:185-220, 1989.

49. Lingerl JB, Orlowski J, Shull MM, Price EM. Molecular genetics of Na,K-ATPase. Prog Nucleic Acids Res Mol Biol 38:37-89, 1990.

50. Young RM, Lingrel JB. Tissue distribution of mRNAs encoding the α isoform and β subunit of rat Na^+,K^+-ATPase. Biochem Biophys Res Commun 145:52-58, 1987.

51. Orlowski J, Lingrel JB. Tissue specific and developmental regulation of rat Na,K-ATPase catalytic alpha isoform and beta subunit mRNAs. J Biol Chem 263:10436-10442, 1988.

52. McDonough AA, Geering K, Farley RA. The sodium pump needs beta subunit. FASEB J 4:1598-1605, 1990.

53. Geering K. The functional role of the beta-subunit in the maturation and intracellular transport of Na,K-ATPase. FEBS Lett 285:189-193, 1991.

54. Noguchi A, Higashi K, Kawamura M. Assembly of the α-subunit of Torpedo californica Na^+-K^+-ATPase with its pre-existing β-subunit in Xenopus oocytes.

Biochim Biophys Acta 1023:247-253, 1990.

55. Kawamura M, Nagano K. Evidence for essential disulfide bonds in the β-subunit of (Na^+-K^+)-ATPase. Biochim Biophys Acta 774:188-192, 1984.

56. Kirley TL. Determination of three disulfide bonds and one free sulfhydryl in the β subunit of (Na^+,K^+)-ATPase. J Biochem Chem 264:7185-7192, 1989.

57. Horisberger JD, Lemas V, Kraehenbuhl JP, Rossier BC. Structure-function relationship of Na^+-K^+-ATPase. Annu Rev Physiol 53:565-584, 1991.

58. Shamraj OI, Melvin D, Lingrel JB. Expression of Na^+,K^+-ATPase isoforms in human heart. Biochem Biophys Res Comm 179:1434-1440, 1991.

59. Sweadner KJ, Herrera VL, Amato S, Moellmann A, Gibbons DK, Repke KR. Immunologic identification of Na^+,K^+-ATPase isoforms in myocardium. Isoform change in deoxycorticosterone acetate-salt hypertension. Circ Res 74:669-678, 1994.

60. Ng YC, Book CB. Expression of Na^+,K^+-ATPase α_1 and α_3 isoforms in adult and neonatal ferret hearts. Am J Physiol 263:H1430-H1436, 1992.

61. Dowell A. Distribution of α_1 and α_2 (Na^+,K^+)-ATPase isoforms between the junctional (t-tubular) and nonjunctional sarcolemmal domains of rat ventricle. Biochem Pharmacol 41:313-315, 1991.

62. Tada M, Yamamoto T, Tonomura Y. Molecular mechanism of active calcium transport by sarcoplasmic reticulum. Physiol Rev 58:1-79, 1978.

63. Grover AK. Ca-pumps in smooth muscle: One in plasma membrane and another in endoplasmic reticulum. Cell Calcium 6:227-236, 1985.

64. Grover AK, Khan I. Calcium pump isoforms: Diversity, selectivity and plasticity. Cell Calcium 13:9-17, 1992.

65. Zarain-Herzberg A, MacLennan DH, Periasamy M. Characterization of rabbit cardiac sarco(endo)-plasmic reticulum Ca^{2+}-ATPase gene. J Biol Chem 265:4670-4677, 1990.

66. Fuji J, Zarain-Herzberg A, Willard HF, Tada M, MacLennan DH. Structure of the rabbit phospholamban gene, cloning of the human cDNA, and assignment of the gene to human chromosome 6. J Biol Chem 266:11669-11675, 1991.

67. Toyofuku T, Zak R. Characterization of cDNA and genomic sequences encoding a chicken phospholamban. J Biol Chem 266:5375-5383, 1991.

68. Toyofuku T, Kurzydlowski K, Tada M, MacLennan DH. Identification of regions in the Ca^{2+}-ATPase of sarcoplasmic reticulum that affect function associated with phospholamban. J Biol Chem 268:2809-2815, 1993.

69. Kranias EG. Regulation of Ca^{2+} transport by protein phosphatase activity associated with cardiac sarcoplasmic reticulum. J Biol Chem 260:11006-11010, 1985.

70. Sasaki T, Inui M, Kimura Y, Kuzuya T, Tada M. Molecular mechanism of regulation of Ca^{2+}-pump ATPase by phospholamban in cardiac sarcoplasmic reticulum. Effects of synthetic phospholamban peptides on Ca^{2+}-pump ATPase. J Biol Chem 267:1674-1679, 1992.

71. MacLennan DH, Campbell KP, Reithmeier RAF. Calsequestrin. In: *Calcium and Cell Function, vol. 4.* Cheng WY (ed). New York: Academic Press, 1983, pp. 151-173.

72. Arai M, Alpert NR, Periasamy M. Cloning and characterization of the gene encoding rabbit cardiac calsequestrin. Gene 109:275-279, 1991.

73. Milner RE, Famulski KS, Michalak M. Calcium binding proteins in the sarcoplasmic/endoplasmic reticulum of muscle and nonmuscle cells. Mol Cell Biochem 112:1-13, 1992.

74. Ostwald TJ, MacLennan DH. Isolation of a high affinity calcium binding protein from sarcoplasmic reticulum. J Biol Chem 249:974-979, 1974.

75. Imanaka-Yoshida K, Amitani A, Ioshii SO, Koyabu S, Yamakado T, Yoshida T. Alterations of expression and distribution of the Ca^{2+}-storing proteins in endo/sarcoplasmic reticulum during differentiation of rat cardiomyocytes. J Mol Cell Cardiol 28:553-562, 1996.

76. Leberer E, Timms BG, Campbell KP, MacLennan DH. Purification, calcium binding properties, and ultrastructural localization of the 53,000- and 160,000 (sarcalumenin)-dalton glycoproteins of the sarcoplasmic reticulum. J Biol Chem 265:10118-10124, 1990.

77. Imagawa T, Smith JS., Coronado R, Campbell KP. Purified ryanodine receptor from skeletal muscle sarcoplasmic reticulum is the Ca^{2+}-permeable pore of the calcium release channel. J Biol Chem 262:16636-16643, 1987.

78. Lai FA, Erickson HP, Rousseau E, Lui QY, Meissner G. Purification and reconstitution of the calcium release channel from skeletal muscle. Nature 331:315-319, 1988.

79. Finkel MS, Shen L, Romeo RC, Oddis CV, Salama G. Radioligand binding and inotropic effects of ryanodine in the cardiomyopathic Syrian hamster. J Cardiovasc Pharmacol 19:610-617, 1992.

80. McKay RG, Pfeffer MA, Pasternak RC, Markis JE, Come PC, Nakao S, Alderman JD, Ferguson JJ, Safian RD, Grossman W. Left ventricular remodeling after myocardial infarction: a corollary to infarct expansion. Circulation 74:693-702, 1986.

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

82. Katz AM. Effects of ischemia on the contractile processes of heart muscle. Am J Cardiol 32:456-460, 1973.

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

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

85. 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 118:484-492, 1985.

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

87. Sabbah HN, Goldstein S. Ventricular remodeling: Consequences and therapy. Europ

Heart J 14 (suppl C):24-29, 1993.

88. Gwathmey JK, Copelas L, MacKinnon R, Schone FJ, Grossman W, Morgan JP. Abnormal intracellular calcium handling in myocardium from patients with end-stage heart failure. Circ Res 61:70-76, 1987.

89. Dixon IMC, Hata T, Dhalla NS. Sarcolemmal calcium transport in congestive heart failure due to myocardial infarction in rats. Am J Physiol 262:H1378-H1394, 1992.

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

91. Limas CJ, Olivari MT, Goldenberg IF, Levine TB, Benditt DG, Simon A. Calcium uptake by cardiac sarcoplasmic reticulum in human dilated cardiomyopathy. Cardiovasc Res 21:601-605, 1987.

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

93. Nagai R, Zarain-Herzberg A, Brandl CJ, Fujii J, Tada M, MacLennan DH, Alpert NR, Periasamy M. Regulation of myocardial Ca^{2+}-ATPase and phospholamban mRNA expression in response to pressure overload and thyroid hormone. Proc Natl Acad Sci USA 86:2966-2970, 1989.

94. Matsui H, MacLennan DH, Alpert N, Periasamy M. Sarcoplasmic reticulum gene expression in pressure overload-induced cardiac hypertrophy in rabbit. Am J Physiol 268:C252-C258, 1995.

95. Kiss E, Ball NA, Kranias EG, Walsh RA. Differential changes in cardiac

phospholamban and sarcoplasmic reticular Ca^{2+}-ATPase protein levels. Effect on Ca^{2+} transport and mechanics in compensated pressure-overload hypertrophy and congestive heart failure. Circ Res 77:759-764, 1995.

96. de la Bastie D, Levitsky D, Rappaport L, Mercadier JJ, Marotte F, Wisnewsky C, Brovkovich V, Schwartz K, Lompré 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 66:554-564, 1990.

97. Arai M, Suzuki T, Nagai R. Sarcoplasmic reticulum genes are upregulated in mild cardiac hypertrophy but downregulated in severe cardiac hypertrophy induced by pressure overload. J Mol Cell Cardiol 28:1583-1590, 1996.

98. Feldman AM, Weinberg EO, Ray PE, Lorell BH. Selective changes in cardiac gene expression during compensated hypertrophy and the transition to cardiac decompensation in rats with chronic aortic banding. Circ Res 73:184-192, 1993.

99. 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 72:463-469, 1993.

100. Arai MK, Otsu T, MacLennan DH, Alpert NR, Periasamy M. Effect of thyroid hormone on the expression of mRNA encoding sarcoplasmic reticulum proteins. Circ Res 69:266-276, 1991.

101. Lompré AM, de la Bastie D, Boheler KR, Schwartz K. Characterization and

expression of the rat heart sarcoplasmic reticulum Ca^{2+} ATPase mRNA. FEBS Lett 249:35-41, 1989.

102. Takahashi T, Schunkert H, Isoyama H, Wei JY, Nadal-Ginard B, Grossman W, Izumo S. Age-related differences in the expression of proto-oncogene and contractile protein genes in response to pressure overload in the rat myocardium. J Clin Invest 89:939-946, 1992.

103. Schwinger RH, Böhm M, Schmidt U, Karczewski P, Bavendiek U, Flesch M, Krause EG, Erdmann E. Unchanged protein levels of SERCA2 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 92:3220-3228, 1995.

104. 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 163/164:285-290, 1996.

105. Nirasawa Y, Akera T. Pressure-induced cardiac hypertrophy: Changes in Na,K-ATPase and glycoside actions in cats. Europ J Pharmacol 137:77-83, 1987.

106. Kim CH, Fan TH, Kelly PF, Himura Y, Delehanty JM, Hang CL, Liang CS. Isoform-specific regulation of myocardial Na,K-ATPase α-subunit in congestive heart failure. Role of norepinephrine. Circulation 89:313-320, 1994.

107. Charlemagne D, Orlowski J, Oliviero P, Rannou F, Sainte-Beuve C, Swynghedauw B, Lane LK. Alteration of Na,K-ATPase subunit mRNA and protein in hypertrophied

rat heart. J Biol Chem 269:1541-1547, 1994.

108. Shamraj OI, Grupp IL, Grupp G, Melvin D, Gradoux N, Kremers W, Lingrel JB, De Pover A. Characterization of Na/K-ATPase, its isoforms, and the inotropic response to ouabain in isolated failing human hearts. Cardiovasc Res 27:2229-2237, 1993.

109. Silver LH, Houser SR. Decreased sodium-potassium pump activity in isolated hypertrophied feline ventricular myocytes. Life Sciences 37:607-615, 1985.

110. Spinale FG, Clayton C, Tanaka R, Fulbright BM, Mukherjee R, Schulte BA, Carwford FA, Zile MR. Myocardial Na^+,K^+-ATPase in tachycardia induced cardiomyopathy. J Mol Cell Cardiol 24:277-294, 1992.

111. Studer R, Reinecke H, Bilger J, Eschehagen T, Böhm 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 75:443-453, 1994.

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

113. Dhalla NS, Dixon IMC, Beamish RE. Biochemical basis of heart function and contractile failure. J Appl Cardiol 6:7-30, 1991.

114. Dhalla NS, Afzal N, Beamish RE, Naimark B, Takeda N, Nagano M. Pathophysiology of cardiac dysfunction in congestive heart failure. Can J Cardiol 9:873-887, 1993.

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

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

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

118. Linck B, Boknik P, Eschenhagen T, Maller FU, Neumann J, Nose M, Jones LR, Schmitz W, Scholz H. Messenger RNA expression and immunological quantification of phospholamban and SR Ca^{2+}-ATPase in failing and nonfailing human hearts. Cardiovasc Res 31:625-632, 1996.

119. Mercadier JJ, Lompré 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 85:305-309, 1990.

120. Brillantes AM, Allen P, Takahashi T, Izumo S, Marks AR. Differences in cardiac calcium release channel (ryanodine receptor) expression in myocardium from patients with end-stage heart failure caused by ischemic versus dilated cardiomyopathy. Circ Res 71:18-26, 1992.

121. Tigerstedt R, Bergman PG. Niere and Kreislauf. Skand Arch Physiol 718:223-271, 1898.

122. Goldblatt H, Lynch J, Hanzal RF, Summerville WW. Studies on experimental

hypertension. 1. The production of persistent elevation of systolic blood pressure by means of renal ischemia. J Exp Med 59:347-379, 1934.

123. Ferguson RK, Brunner HR, Turini GA, Gavras H, McKinstry DN. A specific orally active inhibitor or angiotensin converting enzyme in man. Lancet 1:775-778, 1977.

124. Gavras H, Brunner HR, Turini GA, Kershaw GR, Tifft GP, Cuttelod S, Gavras I, Vukovich RA, McKinstry DN. Antihypertensive effect of the oral angiotensin-converting enzyme inhibitor SQ 14225 in man. N Engl J Med 298:991-995, 1978.

125. Heinrikson RL, Poorman RA. The biochemistry and molecular biology of recombinant human renin and prorenin. In: *Hypertension: Pathophysiology, Diagnosis and Management.* Laragh JH, Brenner BM (eds). New York: Raven Press, 1990, pp 1179-1196.

126. Sigmund CD, Jones CA, Kane CM, Wu C, Lang JA, Gross KW. Regulated tissue- and cell-specific expression of the human renin gene in transgenic mice. Circ Res 70:1070-1079, 1992.

127. Gomez RA, Chevalier RL, Carey RM, Peach MJ. Molecular biology of the renal renin angiotensin system. Kidney International 38 (Suppl 30):S18-S23, 1990.

128. Clauer E, Gaillard I, Li W, Corvol P. Regulation of angiotensinogen gene. Am J Hypertens 2:403-410, 1989.

129. Ehlers MRW, Riordan JF. Angiotensin-converting enzyme: Biochemistry and molecular biology. In: *Hypertension: Pathophysiology, Diagnosis and Management.* Laragh JH, Brenner BM (eds). New York: Raven Press, 1990, pp 1217-1231.

130. Skidgel RA, Erdos EG. The broad substrate specificity of human angiotensin I converting enzyme. Clin Exp Hypertens 9A:243-259, 1987.

131. Corvol P, Michaud A, Soubrier F, Williams TA. Recent advances in knowledge of the structure and function of the angiotensin I converting enzyme. J Hypertens 13 (Suppl 3):S3-S10, 1995.

132. Soubrier F, Alhenc-Gelas F, Hubert C, Allegrine JM, Tregera G, Carvol P. Two putative active centres in human angiotensin I-converting enzyme revealed by molecular cloning. Proc Natl Acad Sci USA 85:9386-9390, 1988.

133. Bernstein KE, Martin BM, Bernstein EA, Linton J, Striker L, Striker G. The isolation of angiotensin-converting enzyme cDNA. J Biol Chem 263:11021-11024, 1988.

134. Rigat B, Hubert C, Corvol P, Soubrier F. PCR detection of the insertion/deletion polymorphism of the human angiotensin I converting enzyme gene (DCPI). Nucleic Acid Res 20:1433, 1992.

135. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting gene accounting for half the variance of serum enzyme levels. J Clin Invest 86:1343-1346, 1990.

136. Triet L, Kee F, Poirier O, Nicaud V, Lecerf L, Evans A, Cambou J-P, Arveiler D, Luc G, Amouyel P. Deletion polymorphism in angiotensin-converting enzyme gene associated with parental history of myocardial infarction. Lancet 341:991-993, 1993.

137. Cambien F, Poirier O, Lecerf L, Evans A, Cambou J-P, Arveiler D, Luc G, Bard J-M, Bara L, Ricard S, Tiret L, Amouyel P, Alhenc-Gelas F, Soubrier F. Deletion

polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. Nature 359:641-644, 1992.

138. Samani NJ, Thompson JR, O'Toole L, Channer K, Woods KL. A meta-analysis of the association of the deletion allele of the angiotensin-converting enzyme gene with myocardial infarction. Circulation 94:708-712, 1996.

139. Dzau VJ, Sasamura H, Hein L. Heterogeneity of angiotensin synthetic pathways and receptor subtypes: Physiological and pharmacological implications. J Hypertens 11 (Suppl 3):S13-S18, 1993.

140. Dzau VJ. Multiple pathways of angiotensin production in the blood vessel wall: Evidence, possibilities and hypotheses. J Hypertens 7:933-936, 1989.

141. Boucher R, Asselin JH, Genest J. A new enzyme leading to direct formation of Ang II. Circ Res 34 (Suppl 1):1203-1209, 1974.

142. Liao Y, Husain A. The chymase-angiotensin system in humans: Biochemistry, molecular biology and potential role in cardiovascular diseases. Can J Cardiol 11 (Suppl F):13F-19F, 1995.

143. Urata H, Healy B, Stewart RW, Bumpus FM, Husain A. Ang II-forming pathways in normal and failing human hearts. Circ Res 66:883-890, 1990.

144. Urata H, Nishimura H, Ganten D. Mechanisms of Ang II formation in humans. Eur Heart J 16 (Suppl N):79-85, 1995.

145. Schunkert H, Ingelfinger JR, Hirsch AT, Pinto Y, Jacob H, Dzau VJ. Feedback regulation of angiotensin converting enzyme activity and mRNA levels by Ang II.

Circ Res 72:312-318, 1993.

146. Biollaz J, Brunner HR, Gavras I, Waeber B, Gavras H. Antihypertensive relationship to evaluate efficacy of converting enzyme blockade. J Cardiovasc Pharmacol 4:966-972, 1982.

147. Danilov SM, Faerman AI, Printseva TO, Martynov AV, Sakharov IY, Trakht IN. Immunohistochemical study of angiotensin-converting enzyme in human tissues using monoclonal antibodies. Histochemistry 87:487-490, 1987.

148. Urata H, Boehm KD, Phillip A, Kinoshita A, Gabrovsek J, Bumpus FM, Husain A. Cellular localization and regional distribution of a major Ang II forming chymase in the heart. J Clin Invest 91:1269-1281, 1993.

149. Chiu AT, Herblin WF, McCall DE, Ardecky RJ, Carini DJ, Duncia JV, Pease LJ, Wong PC, Wexler RR, Johnson AL. Identification of Ang II receptor subtypes. Biochem Biophys Res Commun 165:196-203, 1989.

150. Whitebread S, Mele M, Kamber B, de Gasparo M. Preliminary biochemical characterization of two Ang II receptor subtypes. Biochem Biophys Res Commun 163:284-291, 1989.

151. Clauser E, Curnow KM, Davies E, Conchon S, Teutsch B, Vianello B, Monnot C, Corvol P. Ang II receptors: Protein and gene structures, expression and potential pathological involvement. Eur J Endocrinol 134:403-411, 1996.

152. Szpirer C, Riviere M, Szpirer J, Levan G, Guo DF, Iwai N, Inagami T. Chromosomal assignment of human and rat hypertension candidate genes: Type 1 Ang II receptor

genes and the SA gene. J Hypertens 11:919-925, 1993.

153. Dzau VJ. Molecular biology of Ang II biosynthesis and receptors. Can J Cardiol 11 (Suppl F):21F-26F, 1995.

154. Lin SY, Goodfriend TL. Angiotensin receptors. Am J Physiol 218:1319-1328, 1970.

155. Regitz-Zagrosek V, Auch-Schwelk W, Neuss M, Fleck E. Regulation of the angiotensin receptor subtypes in cell cultures, animal models and human diseases. Eur Heart J 15 (Suppl D):92-97, 1994.

156. Della-Bruna R, Ries S, Himmelstoss C, Kurtz A. Expression of cardiac Ang II AT_1 receptor genes in rat hearts is regulated by steroids but not by Ang II. J Hypertens 13:763-769, 1995.

157. Dzau VJ, Mukoyama M, Pratt RE. Molecular biology of angiotensin receptors: Target for drug research? J Hypertens 12 (Suppl 2):S1-S5, 1994.

158. Murphy TJ, Alexander RW, Griendling KK, Runge MS, Bernstein KE. Isolation of a cDNA encoding the vascular type-1 angiotensin receptor. Nature 351:233-236, 1991.

159. van Heugten HAA, Eskildsen YEG, de Jonge HW, Bezstarosti K, Lamers JMJ. Phosphoinositide-generated messengers in cardiac signal transduction. Mol Cell Biochem 157:5-14, 1996.

160. Duff JL, Marrero MB, Paxton WG, Schieffer B, Bernstein KE, Berk BC. Ang II signal transduction and the mitogen-activated protein kinase pathway. Cardiovasc Res 30:511-517, 1995.

161. Inagami K, Yamano Y, Bardhan S, Chaki S, Guo DF, Ohyama K, Kambayashi Y. Cloning, expression and regulation of Ang II receptors. Adv Exp Med Biol 377:311-317, 1995.

162. Sadoshima JI, Izumo S. Signal transduction pathways of angiotensin II induced c-*fos* gene expression in cardiac myocytes in vitro. Roles of phospholipase-derived second messengers. Circ Res 73:424-438, 1993.

163. Marrero MB, Schieffer B, Paxton WG, Duff JL, Berk BC, Bernstein KE. The role of tyrosine phosphorylation in Ang II-mediated intracellular signalling. Cardiovasc Res 30:530-536, 1995.

164. Yamada T, Horiuchi M, Dzau VJ. Ang II type 2 receptor mediates programmed cell death. Proc Natl Acad Sci USA 93:156-160, 1996.

165. Kijima K, Matsubara H, Murasawa S, Maruyama K, Ohkubo N, Mori Y, Inada M. Regulation of angiotensin type 2 receptor gene by the protein kinase C-calcium pathway. Hypertension 216:359-366, 1996.

166. Jin M, Wilhelm MJ, Lang RE, Unger T, Lindpaintner K, Ganten D. Endogenous tissue renin-angiotensin system. Am J Med 84 (Suppl 3A):28-36, 1988.

167. Johnston CI. Renin-angiotensin system: A dual tissue and hormonal system for cardiovascular control. J Hypertens 10 (Suppl 7):S13-S26, 1992.

168. Danser AHJ. Local renin-angiotensin system. Mol Cell Biochem 157:211-216, 1996.

169. Lindpaintner K, Ganten D. The cardiac renin-angiotensin system: An appraisal of present experimental and clinical evidence. Circ Res 68:905-921, 1991.

170. Dzau VJ. Cardiac renin-angiotensin system: Molecular and functional aspects. Am J Med 84 (Suppl 3A):22-27, 1988.

171. Paul M, Bachmann J, Ganten D. The tissue renin-angiotensin system in cardiovascular disease. Trends Cardiovasc Med 2:94-99, 1992.

172. Campbell DJ. Circulating and tissue renin-angiotensin systems. J Clin Invest 79:1-6, 1987.

173. Dzau VJ, Re RN. Evidence for the renin in the heart. Circulation 73 (Suppl 2):S33-S38, 1987.

174. Nakayama K, Tanata T, Nakanishi S. Tissue distribution of rat angiotensinogen mRNA and structure analysis of its heterogeneity. J Biol Chem 261:319-323, 1986.

175. Campbell DJ, Habener JF. The angiotensinogen gene is expressed and differentially regulated in multiple tissues of the rat. J Clin Invest 78:31-39, 1986.

176. Kunapuli SP, Kumar A. Molecular cloning of human angiotensinogen cDNA and evidence for the presence of its mRNA in the rat heart. Circ Res 60:786-790, 1987.

177. Sawa H, Tokuchi F, Mochizuki N, Mochizuki N, Endo Y, Furuta Y, Shinohara T, Takada A, Kawaguchi H, Yasuda H, Nagashima K. Expression of the angiotensinogen gene and localization of its protein in the human heart. Circulation 86:138-146, 1992.

178. von Lutterotti N, Catanzaro DF, Sealeay JE, Laragh JH. Renin is not synthesized by cardiac and extrarenal vascular tissues. A review of experimental evidence. Circulation 89:458-470, 1994.

179. Lindpaintner K, Jin M, Niedermajer N, Wilhelm MJ, Ganten D. Cardiac angiotensinogen and its local activation in the isolated perfused beating heart. Circ Res 67:564-573, 1990.

180. Yamada H, Fabris B, Allen AM, Jackson B, Johnston CI, Mendelsohn FAO. Localization of angiotensin converting enzyme in rat heart. Circ Res 68:141-149, 1991.

181. Sun Y, Ratajska A, Zhou G, Weber KT. Angiotensin converting enzyme and myocardial fibrosis in the AT receiving Ang II or aldosterone. J Lab Clin Med 122:395-403, 1993.

182. Sun Y, Cleutjens JPM, Diaz-Arias AA, Weber KT. Cardiac angiotensin converting enzyme and myocardial fibrosis in the rat. Cardiovasc Res 28:1423-1432, 1994.

183. Hoit BD, Shao Y, Kinoshita A, Kinoshita A, Gabel M, Husain A, Walsh RA. Effects of Ang II generated by an angiotensin-converting enzyme independent pathway on left ventricular performance in the conscious baboon. J Clin Invest 95:1519-1527, 1995.

184. Baker KM, Campanile CP, Trachte GJ, Peach MJ. Identification of the rabbit Ang II myocardial receptor. Circ Res 54:286-293, 1984.

185. Lokuta AJ, Cooper C, Caa ST, Wang HE, Rogers TB. Ang II stimulates the release of phospholipid-derived second messengers through multiple receptor subtypes in heart cells. J Biol Chem 269:4832-4838, 1994.

186. Koch-Weser J. Nature of the inotropic action of angiotensin on the ventricular

myocardium. Circ Res 16:239-237, 1965.

187. Ishihata A, Endoh M. Species-related differences in inotropic effects of Ang II in mammalian ventricular muscle: Receptors, subtype and phosphoinositide hydrolysis. Br J Pharmacol 114:447-453, 1995.

188. Yamazaki T, Komuro I, Shiojimo I, Yazaki Y. The renin-angiotensin system and cardiac hypertrophy. Heart 76 (Suppl 3):33-35, 1996.

189. Allen I, Cohen NM, Dhallan RS, Gaa ST, Lederer WJ, Rogers TB. Ang II increases spontaneous contractile frequency and stimulates calcium current in cultures neonatal rat heart myocytes: Insight into underlying biochemical mechanisms. Circ Res 62:524-534, 1988.

190. Freer R, Pappano A, Peach M, Ning K, McLean M, Vogel S, Sperelakis N. Mechanism for the positive inotropic effect of Ang II on isolated cardiac muscle. Circ Res 39;178-183, 1976.

191. Bonnardeaux JL, Regoli D. Action of angiotensin and analogues on the heart. Can J Physiol Pharmacol 52:50-60, 1974.

192. Cross RB, Chalk J, South M, Liss B. The action of angiotensin on the isolated cat heart. Life Sci 29:903-908, 1981.

193. Zhang J, Pfaffendorf M, van Zwieten PA. Hemodynamic effects of Ang II and the influence of angiotensin receptor antagonists in pithed rabbits. J Cardiovasc Pharmacol 25:724-731, 1995.

194. Ikenouchi H, Barry WH, Bridge JHB, Weinberg EO, Apstein CS, Lorell BH. Effects

of Ang II on intracellular Ca^{2+} and pH in isolated beating rabbit hearts and myocytes loaded with the indicator indo-1. J Physiol 48: 203-215, 1994.

195. Huang H, Li P, Hamby CV, Reiss K, Meggs LG, Anversa P. Alteration in Ang II receptor mediated signal transduction shortly after coronary artery constriction in the rat. Cardiovasc Res 28:1564-1573, 1994.

196. Lkeda U, Maeda Y, Kawahara Y, Yokoyama M, Shimada K. Ang II augments cytosine-stimulated nitric oxide synthesis in rat cardiac myocytes. Circulation 92: 2683-2689, 1995.

197. Zhu YC, Zhu YZ, Spitznagel H, Gohlke P, Unger T. Substrate metabolism, hormone interaction, and angiotensin-converting enzyme inhibitors in left ventricular hypertrophy. Diabetes 45 (Suppl 1): S59-S65, 1996.

198. Schlueter W, Keilani T, Batlle DC. Metabolic effects of converting enzyme inhibitors: Focus on the reduction of cholesterol and lipoprotein (a) by fosinopril. Am J Cardiol 72:37H-44H, 1993.

199. Zimmermann B. Adrenergic facilitation by angiotensin: Does it serve a physiologic function? Clin Sci 60:343-348, 1981.

200. Xiang J, Linz W, Becker H, Ganten D, Lang RE, Scholkens B, Unger T. Effects of converting enzyme inhibitors ramipril and enalapril on peptide action and sympathetic neurotransmission in the isolated heart. Eur J Pharmacol 113:215-223, 1985.

201. Eckberg DL, Drabinsky M, Braunwald E. Defective cardiac parasympathetic control in patients with heart disease. N Engl J Med 285:877-883, 1971.

heart cells. Am J Physiol 259:H610-H618, 1990.

211. Greenen DL, Malhotra A, Scheuer J. Ang II increases cardiac protein synthesis in adult rat heart. Am J Physiol 265:H238-H243, 1993.

212. van Krimpen C, Smits JFM, Cleutjens JPM, Debets JJM, Schoemaker RG, Struyker Boudier HAJ, Bosman FT, Daemen MJAP. DNA synthesis in the non-infarcted cardiac interstitium after left coronary artery ligation in the rat: Effects of captopril. J Mol Cell Cardiol 23:1245-1253, 1991.

213. Fisher SA, Absher M. Norepinephrine and ANG II stimulate secretion of TGF-β by neonatal rat cardiac fibroblasts in vitro. Am J Physiol 268:C910-C917, 1995.

214. Kato H, Suzuki H, Tajima S, Ogata Y, Tominaga T, Sato A, Saruta T. Ang II stimulates collagen synthesis in cultured vascular smooth muscle cells. J Hypertens 9:17-22, 1991.

215. Schorb W, Booz GW, Dostal DE, Conrad KM, Chang KC, Baker KM. Ang II is mitogenic in neonatal rat cardiac fibroblasts. Circ Res 72:1245-1254, 1993.

216. Morgan HE, Baker KM. Cardiac hypertrophy: Mechanical, neural and endocrine dependencies. Circulation 83:13-26, 1991.

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

218. Dhalla NS, Beamish RE. Cellular and molecular approaches for understanding heart failure. Prairie Med J 65:117-120, 1995.

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

220. Harrap SB, Dominiczak AF, Fraser R, Lever AF, Morton JJ, Foy CJ, Watt GCM. Plasma Ang II, predisposition to hypertension and left ventricular size in healthy young adults. Circulation 93:1148-1154, 1996.

221. Ohta K, Kim S, Wanibuchi H, Ganten D, Iwao H. Contribution of local renin-angiotensin system to cardiac hypertrophy, phenotypic modulation, and remodeling in TGR(mREN2)27 transgenic rats. Circulation 94:785-791, 1996.

222. Bader M, Zhao Y, Sander M, Lee MA, Bachmann J, Böhm M, Djavidani B, Peters J, Mullins JJ, Ganten D. Role of tissue renin in the pathophysiology of hypertension in TGR(mREN2)27 rats. Hypertension 19:681-686, 1992.

223. Böhm M, Lee MA, Krauts R, Kim S, Schinke M, Djavidani B, Wagner J, Kaling M, Wirnen W, Bader M, Ganten D. Ang II receptor blockade in TGR(mREN2)27: Effect of renin-angiotensin-system gene expression and cardiovascular functions. J Hypertens 13:891-899, 1995.

224. Kawaguchi H, Kitabatake A. Renin-angiotensin system in failing heart. J Mol Cell Cardiol 27:201-209, 1995.

225. Weber KT, Sun Y, Tyagi SC, Cleutjens JPA. Collagen network of the myocardium: Function, structure remodeling and regulatory mechanisms. J Mol Cell Cardiol 26:279-292, 1994.

226. Boluyt MO, O'Neill L, Meredith AL, Bing OHL, Brooks WW, Conrad Ch, Crow MT, Lakatta EG. Alterations in cardiac gene expression during the transition from stable hypertrophy to heart failure: Marked upregulation of gene encoding extracellular matrix components. Circ Res 75:23-32, 1994.

227. Kim S, Ohta K, Hamaguchi A, Omura T, Yukimura T, Miura K, Inada Y, Ishimura Y, Chatani F, Iwao H. Ang II type 1 receptor antagonists inhibit the gene expression of transforming growth factor-β1 and extracellular matrix in cardiac and vascular tissues of hypertensive rats. J Pharmacol Exp Ther 273:509-515, 1995.

228. Iwani K, Ashizawa N, Do YS, Graf K, Hsueh W. Comparison of ANG II with other growth factors on EGR-I and matrix gene expression in cardiac fibroblast. Am J Physiol 270:H2100-H2107, 1996.

229. Hsueh WA, Do YS, Anderson PW, Law RE. Ang II in cell growth and matrix production. Adv Exp Med Biol 377: 217-223, 1995.

230. Brilla CG, Zhou G, Rupp H, Maisch B, Weber KT. Role of Ang II and prostaglandin E2 in regulating cardiac fibroblast collagen turnover. Am J Cardiol 76:8D-13D, 1995.

231. Crawford D, Chobanian AV, Brecher P. Ang II induced fibronectin expression associated with cardiac fibrosis in the rat. Circ Res 74:727-739, 1994.

232. Dostal DE, Booz GW, Baker KM. Ang II signaling pathways in cardiac fibroblasts: Conventional versus novel mechanisms in mediating cardiac growth and function. Mol Cell Biochem 157:15-21, 1996.

233. Sadoshima JI, Xu Y, Slayter HS, Izumo S. Autocrine release of Ang II mediated stretch-induced hypertrophy of cardiac myocytes in vitro. Cell 75:977-984, 1993.

234. Kent RL, McDermott PJ. Passive load and Ang II evoke differential responses of gene expression and protein synthesis in cardiac myocytes. Circ Res 78:829-838, 1996.

235. Reiss K, Capasso JM, Huang HE, Meggs LG, Li P, Anversa P. Ang II receptors, c-*myc*, and c-*jun* in myocytes after myocardial infarction and ventricular failure. Am J Physiol 264:H760-H769, 1993.

236. Lee AA, Dillmann WH, McCulloch AD, Villarreal FJ. Ang II stimulates the autocrine production of transforming growth factor-β1 in adult rat cardiac fibroblasts. J Mol Cell Cardiol 27:2347-2357, 1995.

237. Du J, Meng XP, Delafontaine P. Transcriptional regulation of the insulin-like growth factor-I receptor gene: Evidence for protein kinase C-dependent and independent pathways. Endocrinology 137:1378-1384, 1996.

238. Everett AD, Tufro-McReddie A, Fisher A, Gomez RA. Angiotensin receptor regulates cardiac hypertrophy and transforming growth factor-β_1 expression. Hypertension 23:587-592, 1994.

239. Booz GW, Taher MM, Baker KM, Singer HA. Ang II induces phosphatidic acid formation in neonatal rat fibroblasts: evaluation of the roles of phospholipases C and D. Mol Cell Biochem 141:135-143, 1994.

240. Booz GW, Dostal DE, Singer HA, Baker KM. Involvement of protein kinase C and

Ca^{2+} in Ang II-induced mitogenesis of cardiac fibroblasts. Am J Physiol 267:C1308-C1318, 1994.

241. Bogoyevitch MA, Glennon PE, Andersson MB, Clerk A, Lazou A, Marshall CJ, Parker PJ, Sugden PH. Endothelin-I and fibroblast growth factors stimulate the mitogen-activated protein kinase signaling cascade in cardiac myocytes. The potential role of the cascade in the integration of two signaling pathways leading to myocyte hypertrophy. J Biol Chem 269:1110-1119, 1994.

242. Ishiye M, Umemura K, Uematsu T, Nakashima M. Effects of losartan, an Ang II antagonist, on the development of cardiac hypertrophy to volume overload. Biol Pharm Bull 18:700-704, 1995.

243. Federico Pieruzzi F, Xaid A, Abassi ZA, Keiser HR. Expression of renin-angiotensin system components in the heart, kidneys, and lungs of rats with experimental heart failure. Circulation 92:3105-3112, 1995.

244. Schunkert H, Dzau VJ, Tong SS, Hirsch AT, Apstein CS, Lorell BH. Increased rat cardiac angiotensin converting enzyme activity and mRNA levels in pressure overload left ventricular hypertrophy: Effects on coronary resistance, contractility and relaxation. J Clin Invest 86:1913-1920, 1990.

245. Iwai N, Shimoike H, Kinoshita M. Cardiac renin-angiotensin system in the hypertrophied heart. Circulation 92:2690-2696, 1995.

246. Sun Y, Weber KT. Cells expressing Ang II receptors in fibrous tissues of rat heart. Cardiovasc Res 31:518-525, 1996.

247. Zhang X, Dostal DE, Reiss K, Cheng W, Kajstura J, Li P, Huang H, Sonnenblick EH,

Meggs L, Baker KM, Anversa P. Identification and activation of autocrine renin-angiotensin system in adult ventricular myocytes. Am J Physiol. 269:H1791-H1802, 1995.

248. Hirsh AT, Talsness CE, Schunlert H, Paul M, Dzau VJ. Tissue-specific activation of cardiac angiotensin converting enzyme in experimental heart failure. Circ Res 69:475-482, 1991.

249. Nio Y, Matsubara H, Murasawa S, Kanasaki M, Inada M. Regulation of gene transcription of Ang II receptor subtypes in myocardial infarction. J Clin Invest 95:46-54, 1995.

250. Tang SS, Diamant D, Rogg H, Schunkert H, Lorell BH, Ingelfinger JR. Rat hearts contain Ang II (ANGII) receptors that are downregulated and differentially expressed during hypertrophy. Hypertension 20:418, 1992 (Abstract).

251. Nishimura J, Kobayashi S, Chen X, Shikasho T, Kanaide H. Ang II receptor mRNA is regulated by Ang II: Possible involvement of protein kinase C in receptor downregulation. Circulation 86 (Suppl 1):I-289, 1992 (Abstract).

252. Suzuki J, Matsubara H, Urakami M, Inada M. Rat Ang II (type 1) receptor mRNA regulation and subtype expression in myocardial growth and hypertrophy. Circ Res 73:439-447, 1993.

253. Meggs LG, Coupet J, Huang H, Cheng W, Li P, Capes JM, Homcy CJ, Anversa P. Regulation of Ang II receptors on ventricular myocytes after myocardial infarction in rats. Circ Res 72:1149-1162, 1993.

254. Zagrosek VR, Friedel N, Heymann A, Bauer P, Rolfs A, Steffen C, Hildebrandt A, Whether R, Fleck E. Regulation, chamber localization, and subtype distribution of Ang II receptors in human hearts. Circulation 91:1461-1471, 1995.

255. Schunker H, Tang SS, Litwin SE, Diamant D, Riegger G, Dzau VJ, Ingelfinger JR. Regulation of intrarenal and circulating renin-angiotensin systems in severe heart failure in the rat. Cardiovasc Res 27:731-735, 1993.

256. Raynolds MV, Bristow MR, Bush EW, Abraham WT, Lowes BD, Zisman LS, Taft CS, Perryman MB. Angiotensin-converting enzyme DD genotype in patients with ischemic or idiopathic dilated cardiomyopathy. Lancet 342:1073-1075, 1993.

257. Pinto YM, van Gilst WH, Kingma JH, Schunkert H. Captopril and thrombolysis study investigators. Deletion type allele of the angiotensin-converting enzyme gene is associated with progressive ventricular dilation after anterior myocardial infarction. J Am Coll Cardiol 25:1622-1626, 1995.

258. Arbustini E, Grasso M, Fasani R, Klersy C, Diegoli M, Porcu E, Banchieri N, Fortina P, Danesino C, Specchia G. Angiotensin converting enzyme gene deletion allele is independently and strongly associated with coronary atherosclerosis and myocardial infarction. Br Heart J 74:584-591, 1995.

259. Gharavi AG, Lipkowitz MS, Diamond JA, Jhang JS, Phillips RA. Deletion polymorphism of the angiotensin-converting enzyme gene is independently associated with left ventricular mass and geometric remodeling in systemic hypertension. Am J Cardiol 77:1315-1319, 1996.

260. Andersson B, Sylvén C. The DD genotype of the angiotensin-converting enzyme gene is associated with increased mortality in idiopathic heart failure. J Am Coll Cardiol 28:162-167, 1996.

261. Schunkert H, Hense HW, Holmer SR, Stender M, Perz S, Kell U, Lorell BH, Riegger GAJ. Association between a deletion polymorphism of the angiotensin-converting-enzyme gene and left ventricular hypertrophy. N Engl J Med 330:1634-1638, 1994.

262. Lindpaintner K, Lee M, Larson MG, Rao VS, Pfeffer MA, Ordovas O, Schaefer EJ, Wilson AF, Wilson PWF, Vasan RS, Myers RH, Levy D. Absence of association or genetic between the angiotensin-converting-enzyme gene. N Engl J Med 334:1023-1028, 1996.

263. Ondertti MA, Rubin B, Cushman DW. Design of specific inhibitors of angiotensin converting enzyme: New class of orally active antihypertensive agents. Science 196:441-443, 1977.

264. Juggi JS, Berard GK, van Gilst WH. Cardioprotection by angiotensin-converting enzyme (ACE) inhibitors. Can J Cardiology 9:336-352, 1993.

265. Cohen ML. Synthetic and fermentation-derived angiotensin-converting enzyme inhibitors. Ann Rev Pharmacol Toxicol 25:307-323, 1985.

266. Braunwald E. ACE inhibitors - a cornerstone of the treatment of heart failure. N Engl J Med 325:351-353, 1991.

267. Opie LH. Fundamental role of angiotensin-converting enzyme inhibitors in the management of congestive heart failure. Am J Cardiol 75:3F-6F, 1995.

268. Melton DM, Holzgrefe HH, Walker JD, Mukherjee R, Arthur SR, Antoneccio MJ, Koster WH, Spinale FG. Effect of chronic angiotensin-converting enzyme inhibitor on left ventricular and myocyte structure and function during recovery from chronic rapid pacing. J Mol Cell Cardiol 290:697-710, 1997.

269. Raya TE, Gay RG, Aguirre M, Goldman S. Importance of vasodilatation in prevention of left ventricular dilatation after chronic large myocardial infarction in rats: A comparison of captopril and hydrazine. Circ Res 64:330-337, 1989.

270. Mehta PM, Alker KJ, Kloner RA. Functional infarct expansion, left ventricular dilatation, and isovolume in occlusion: A two dimensional echocardiographic study. J Am Coll Cardiol 11:630-636, 1988.

271. Sweet CS. Issues surrounding a local cardiac renin system and the beneficial actions of angiotensin-converting enzyme inhibitors in ischemic myocardium. Am J Cardiol 65:111-113, 1990.

272. Litwin SE, Litwin CM, Raya TE, Warner AL, Goldman S. Contractility and stiffness of noninfarcted myocardium after coronary ligation in rats. Effects of chronic angiotensin converting enzyme inhibition. Circulation 83:1028-1037, 1991.

273. Pfeffer JM, Pfeffer MA, Braunwald E. Influence of chronic captopril therapy on the infarcted left ventricle of the rat. Circ Res 57:84-95, 1985.

274. Lefer AM, Peck RC. Cardioprotective effects of enalapril in acute myocardial ischemia. Pharmacology 29:61-69, 1984.

275. Ertl G, Kloner RA, Alexander W, Braunwald E. Limitation of experimental infarct

size by an angiotensin-converting enzyme inhibitor. Circulation 65:40-48, 1982.

276. Daniell HB, Carson RR, Ballard KD, Tomas GR, Privitera PJ. Effect of captopril on limiting infarct size in conscious dogs. J Cardiovasc Pharmacol 6:1043-1047, 1984.

277. Liang CS, Gavras H, Black J, Sherman LG, Hood WB. Renin-angiotensin system in acute myocardial infarction in dogs. Effects on systemic hemodynamics, myocardial blood flow, segmental myocardial function and infarct size. Circulation 66:1249-1255, 1982.

278. Leddy CL, Wilen M, Francious JA. Effects of a new angiotensin converting enzyme inhibitor, enalapril, in acute and chronic left ventricular failure. J Clin Pharmacol 23:189-198, 1983.

279. Hock CE, Riberiro LGT, Lefer AM. Prevention of ischemic myocardium by a new converting enzyme inhibitor, enalapril acid. Am Heart J 109:222-228, 1985.

280. Jeremic G, Masson S, Luvarà G, Porzio S, Lagrasta C, Riva E, Olivetti G, Latini R. Effects of new angiotensin-converting enzyme inhibitor (idrapril) in rats with left ventricular dysfunction after myocardial infarction. J Cardiovasc Pharmacol 27:347-354, 1996.

281. Pfeffer MA, Braunwald E, Moyé LA, Basta L, Brown EJ, Cuddy TE, Davis BR, Geltman EM, Goldman S, Flaker GC. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction. N Engl J Med 327:669-677, 1992.

282. Emmert SE, Stabilito II, Sweet CS. Acute and subacute hemodynamic effects of

enalaprilat, milrinone and combination therapy in rats with chronic left ventricular dysfunction. Clin Exp Ther Prac A9:297-306, 1987.

283. Fornes P, Richer C, Pussard E, Heudes D, Domergue V, Giudicelli JF. Beneficial effects of trandolapril on experimental induced congestive heart failure in rats. Am J Cardiol 70:43D-51D, 1992.

284. Beermann A, Nyquist O, Höglund C, Jacobsson KA, Näslund U, Jensen-Urstad M. Acute haemodynamic effects and pharmacokinetics of ramipril in patients with heart failure. A placebo controlled three-dose study. Eur J Clin Pharmacol 45:241-246, 1993.

285. Pfeffer MA, Lamas GA, Vaughan DE, Parisi AF, Braunwald E. Effect of captopril on progressive ventricular dilatation after anterior myocardial infarction. N Engl J Med 319:80-86, 1988.

286. Anning PB, Grocott RM, Lewis MJ, Shah AM. Enhancement of left ventricular relaxation in the isolated heart by an angiotensin converting enzyme inhibitor. Circulation 92:2660-2665, 1995.

287. The SOLVD Investigators. Effect of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection. N Engl J Med 327:685-691, 1992.

288. Ambrosio E, Borghi C, Magnani B. For the survival of myocardial infarction long-term evaluation (SMILE) study investigation. The effect of the angiotensin-converting-enzyme inhibitor zofenopril on mortality and morbidity after anterior

myocardial infarction. N Engl J Med 332:80-85, 1995.

289. *ISIS-4* (Fourth international study of infarct survival) collaborative group. ISIS-4: A randomized factorial trial assessing early oral captopril, oral mononitrate, and intravenous magnesium sulphate in 58050 patients with suspected acute myocardial infarction. Lancet 345:669-685, 1995.

290. Swedberg K, Held P, Kjekshus J and CONSENSUS II Investigators. Effects of the early administration of enalapril on mortality in patients with acute myocardial infarction. Results of the Cooperative New Scandinavian Enalapril Survival Study II (CONSENSUS-II). N Engl J Med 327:678-684, 1992.

291. Gruppo italiano per lo studio delia sopravvivenza nell'infarto miocardico. Six-month effects of early treatment with lisinopril and transdermal glyceryl trinitrate singly and together withdrawn six weeks after acute myocardial infarction: The GISSI-3 trial. J Am Coll Cardiol 27:337-344, 1996.

292. Cleland JG, Puri S. How do ACE inhibitor reduce mortality in patients with left ventricular dysfunction with or without heart failure: Remodeling, resetting, or sudden death? Br Heart J 72 (Suppl 3): S81-S86, 1994.

293. Beckwith C, Munger MA. Effect of angiotensin-converting enzyme inhibitor on ventricular remodeling and survival following myocardial infarction. Ann Pharmacotherap 27:755-766, 1993.

294. Pfeffer JM, Fischer TA, Pfeffer MA. Angiotensin-converting enzyme inhibition and ventricular remodeling after myocardial infarction. Ann Rev Physiol 57:805-826,

1995.

295. Goldstein S, Sharov VG, Cook JM, Sabbah HN. Ventricular remodeling: Insights from pharmacologic interventions with angiotensin converting enzyme inhibitors. Mol Cell Biochem 147:51-55, 1995.

296. Kramer CM, Ferrari VA, Rogers WJ, Theobald TM, Nance LM, Axel L, Reichek A. Angiotensin-converting enzyme inhibition limits dysfunction in adjacent noninfarcted regions during left ventricular remodeling. J Am Coll Cardiol 27:211-217, 1996.

297. Gaballa MA, Raya T, Goldman S. Large artery remodeling after myocardial infarction. Am J Physiol 268:H2092-H2103, 1995.

298. Dixon IMC, Ju H, Jassal DS, Peterson DJ. Effect of ramipril and losartan on collagen expression in right and left heart after myocardial infarction. Mol Cell Biochem 165:31-45, 1996.

299. Ball SG, Hall AS, Murray GD. Angiotensin-converting enzyme inhibitors after myocardial infarction: Indications and timing. J Am Coll Cardiol 25 (Suppl 7):42S-46S, 1995.

300. van Gilst WH, Kingma JH, Peels KH, Dambrink JHE, Sutton MSJ. Which patient benefits from early angiotensin-converting enzyme inhibition after myocardial infarction? Results of one-year serial echocardiographic following from the captopril and thrombolysis study (CATS). J Am Coll Cardiol 28:114-121, 1996.

301. Schoemaker RG, Debets JJM, Struyker-Boudier HAJ, Smits JFM. Delayed but not immediate captopril therapy improves cardiac function in conscious rats following

myocardial infarction. J Mol Cell Cardiol 23:187-197, 1991.

302. Cleland JGF, Poole-Wilson PA. ACE inhibitors for heart failure: A question of dose. Br Heart J 72 (Suppl 3):106-110, 1994.

303. Wollert KC, Studer R, von Bülow B, Drexler H. Survival after myocardial infarction in the rat. Role of tissue angiotensin-converting enzyme inhibition. Circulation 90:2457-2467, 1994.

304. Perich RB, Jackson B, Rogerson F, Mendelsohn FAO, Paxton D, Johnston CL. Two binding sites on angiotensin I-converting enzyme: Evidence from radioligand binding studies. Mol Pharmacol 42:286-293, 1992.

305. Dell'Italia LJ, Oparil S. Cardiac renin angiotensin system in hypertrophy and the progression to heart failure. Heart Failure Reviews 1:63-72, 1996.

306. Garcia R, Qing G. Characterization of plasma and tissue atrial natriuretic factor during development of moderate high output heart failure in the rat. Circ Res 27:464-470, 1993.

307. Winkins MR, Settle SL, Stockmann PT, Needleman P. Maximizing the natriuretic effect of endogenous atriopeptin in a rat model of heart failure. Proc Natl Acad Sci USA 87:6465-6469, 1990.

308. Arnal JF, Philippe M, Laboulandine I, Michel JB. Effect of perindopril in rat cardiac volume overload. Am Heart J 126:776-782, 1993.

309. Takeda N, Tanamura A, Iwai T, Kato M, Noma K, Nagano M. Beneficial effect of ACE inhibitor in congestive heart failure. Mol Cell Biochem 129:139-143, 1993.

310. Ruzicka M, Yuan B, Harmsen E, Leenen FHH. The renin-angiotensin system and volume overload-induced cardiac hypertrophy in rats: Effects of angiotensin converting enzyme inhibitor versus Ang II receptor blocker. Circulation 87:921-930, 1993.

311. Ruzicka M, Yuan B, Leenen FHH. Effects of enalapril versus losartan on regression of volume overload-induced cardiac hypertrophy in rats. Circulation 90:484-491, 1994.

312. Garcia R, Bonhomme MC, Diebold S. Captopril treatment does not restore either the renal or the ANF release response during volume expansion in moderate to severe high output heart failure. Cardiovasc Res 28:1533-1539, 1994.

313. Ruzicka M, Keeley FW, Leenen FHH. The renin-angiotensin system and volume overload-induced changes in cardiac collagen and elastin. Circulation 90:1989-1996, 1994.

314. Ruzicka M, Leenen FHH. Relevance of blockade of cardiac and circulatory angiotensin-converting enzyme for the prevention of volume overload-induced cardiac hypertrophy. Circulation 91:16-19, 1995.

315. Ruzicka M, Skarda V, Leenen FHH. Effects of ACE inhibitors on circulating versus cardiac angiotensin II in volume overload induced cardiac hypertrophy in rats. Circulation 92: 3568-3573, 1995.

316. Feldman AM, Weinberg EO, Ray PH, Lorell BH. Selective changes in cardiac gene expression during compensated hypertrophy and the transition to cardiac

decompensation in rats with chronic aortic banding. Circ Res 73:184-192, 1993.

317. Baker KM, Chernin MI, Wixson SK, Aceto JF. Renin angiotensin system involvement in pressure-overload cardiac hypertrophy in rats. Am J Physiol 259:H324-H332, 1990.

318. Kromer EP, Riegger GAJ. Effects of long-term angiotensin converting enzyme inhibition on myocardial hypertrophy in experimental aortic stenosis in the rat. Am J Cardiol 62:161-163, 1988.

319. Linz W, Scholkens BA, Ganten D. Converting enzyme inhibition specifically prevents the development and induced regression of cardiac hypertrophy in rats. Clin Exp Hypertens 11A:1325-1350, 1989.

320. Weinberg EO, Schoen FJ, George D, Kagaya Y, Douglas PS, Litwin SE, Schunkert H, Benedict CR, Lorell BH. Angiotensin-converting enzyme inhibition prolongs survival and modifies the transition to heart failure in rats with pressure overload hypertrophy due to ascending aortic stenosis. Circulation 90:1410-1422, 1994.

321. Litwin SE, Katz SE, Weinberg EO, Lorell BH, Aurigemmma GP, Douglas PS. Serial echocardiographic-Doppler assessment of left ventricular geometry and function in rats with pressure-overload hypertrophy: Chronic angiotensin-converting enzyme inhibition attenuated the transition to heart failure. Circulation 91:2642-2654, 1995.

322. Mohabir R, Young SD, Strosberg AM. Role of angiotensin in pressure overload-induced hypertrophy in rats: Effects of angiotensin-converting enzyme inhibitors, an AT_1 receptor antagonist, and surgical reversal. J Cardiol Pharmacol 23:291-299,

1994.

323. Spinale FG, Holzgrefe HH, Mukherjee R, Hird RB, Walker JD, Arnim-Barker A, Powell JR, Koster WH. Angiotensin-converting enzyme inhibition and the progression of congestive cardiomyopathy: Effects of left ventricular and myocyte structure and function. Circulation 92:562-578, 1995.

324. Ogilvie RI, Zborowska-Sluis D. Captopril attenuates pacing-induced acute heart failure by increasing total vascular capacitance. J Cardiol Pharmacol 22:153-159, 1993.

325. Hirakata H, Fouad-Trazi FM, Bumpus FM, Khosla M, Healy B, Husain A, Urata H, Kumagai H. Angiotensin and the failing heart: Enhanced positive inotropic response to angiotensin I in cardiomyopathic hamster heart in the presence of captopril. Circ Res 66:891-899, 1990.

326. Masutomo K, Makino N, Matuyama T, Shimada T, Yanaga T. Effects of enalapril on the collagen matrix in cardiomyopathic Syrian hamsters (BIO 14.6 and 53.58). Jpn Circ J 60:50-61, 1996.

327. Chopra M, Scott N, McMurray J, McLay J, Bridges A, Smith WE, Belch JJF. Captopril: A free radical scavenger. Br J Clin Pharmacol 27:396-399, 1989.

328. Bagchi D, Prasad R, Das DK. Direct scavenging of free radical by captopril, an angiotensin converting enzyme inhibitor. Biochem Biophys Res Comm 158:52-57, 1989.

329. Ziehut W, Studer R, Laurent D, Kästner S, Allegrini P, Whitebread S, Cumin F,

heart failure by monocrotaline without reducing pulmonary hypertension in rats: Role of preserved myocardial creatine kinase and lactate dehydrogenase isoenzymes. Int J Cardiol 47:225-233, 1995.

344. Zhang X, Xie YW, Nasjletti A, Xu X, Wolin MS, Hintze TH. ACE inhibitor promotes nitric oxide accumulation to modulate myocardial oxygen consumption. Circulation 95:176-182, 1997.

345. Pals DT, Mosucci FD, Sipos F, Denning GSJR. A specific competitive inhibitor of Ang II. Proc Natl Acad Sci USA 67:1624-1630, 1970.

346. Chiu AT, McCall DE, Price WA, Wong PC, Carini DJ, Duncia JV. Non pepitide Ang II receptor antagonists: VII. Cellular and biochemical pharmacology of DuP 753, an orally active antihypertensive agent. J Pharmacol Exp Ther 252:711-718, 1990.

347. Wong PC, Price WA, Chiu AT, Duncia JV, Chrini DJ, Wexler RR. Nonpeptide Ang II receptor antagonists: VIII. Characterization of functional antagonism displayed by DuP 753, an orally active antihypertensive agent. J Pharmacol Exp Ther 252:719-725, 1990.

348. Duncia JV, Carini DJ, Chiu AT, Johnson AL, Price WA, Wong PC, Wexler RR, Timmermans PBMWM. The discovery of DuP 753, a potent, orally active nonpeptide Ang II receptor antagonist. Med Res Rev 12:149-191, 1992.

349. Ji H, Leung M, Zhang Y, Catt KJ, Sandberg K. Differential structure requirements for specific binding of nonpeptide and peptide antagonists to the AT1 angiotensin receptor: Identification of amino acid residues that determine binding of the

antihypertensive drug losartan. J Biol Chem 269:16533-16536, 1994.

350. Raya TE, Fonken SJ, Lee RW, Daugherty S, Goldman S, Wong PC, Timmermans PBMWM, Morkin E. Hemodynamic effects of direct Ang II blockade compared to converting enzyme inhibition in rat model of heart failure. Am J Hypertens 4:334S-340S, 1991.

351. Smits JFM, van Krimpen C, Schoemaker RG, Cleutjens JPM, Daemen MJAP. Ang II receptor blockade after myocardial infarction in rats: Effects of hemodynamics, myocardial DNA synthesis, and interstitial collagen content. J Cardiovasc Pharmacol 20:772-778, 1992.

352. Gottlieb SS, Dickstein KD, Fleck E, Kostis J, Levine TB, LeJemtel T, DeKock M. Hemodynamic and neurohormonal effects of the Ang II antagonist losartan in patients with congestive heart failure. Circulation 88:1602-1609, 1993.

353. Regitz-Zagrosek V, Neuss M, Holzmeister J, Fleck E. Use of Ang II antagonists in human heart failure: Function of the subtype 1 receptor. J Hypertens 13 (Suppl 1):S63-S71, 1995.

354. Crozier I, Ikram H, Awan N, Cleland J, Stephen N, Dickstein K, Frey M, Young J, Klinger G, Makris L, Rucinska E. For the losartan hemodynamic study group. Losartan in heart failure: Hemodynamic effects and tolerability. Circulation 91:691-697, 1995.

355. Kojima M, Shiojima I, Yamazaki T, Komuro I, Zou Y, Wang Y, Mizuno T, Ueki K, Tobe K, Kadowaki T, Nagai R, Yazaki Y. Ang II receptor antagonist TCV-116

induces regression of hypertensive left ventricular hypertrophy in vivo and inhibits the intracellular signaling pathway of stretch-mediated cardiomyocyte hypertrophy in vitro. Circulation 89:2204-2211, 1994.

356. Cheng CP, Suzuki M, Ohte N, Ohno M, Wang ZM, Little WC. Altered ventricular and myocyte response to Ang II in pacing-induced heart failure. Circ Res 78:880-892, 1996.

357. Hantani A, Yoshiyama M, Kim S, Omura T, Toda I, Akioka K, Teragaki M, Takeuchi K, Iwao H, Takeda T. Inhibition by Ang II type 1 receptor antagonist of cardiac phenotypic modulation after myocardial infarction. J Mol Cell Cardiol 27:1905-1914, 1995.

358. Nishikimi T, Yamagishi H, Takeuchi K, Takeda T. An Ang II receptor antagonist attenuates left ventricular dilatation after myocardial infraction in the hypertensive rat. Cardiovasc Res 29:856-861, 1995.

359. Rush JE, Rajfer SI. Theoretical basis for the use of Ang II antagonists in the treatment of heart failure. J Hypertens 11 (Suppl 3):S69-S71, 1993.

360. Campbell DJ, Kladis A, Valentijn AJ. Effects of losartan on angiotensin and bradykinin peptides and angiotensin converting enzyme. J Cardiol Pharmacol 26:233-240, 1995.

361. Dickstein K, Chang P, Willenheimer R, Haunsø S, Remes J, Hall C, Kjekshus J. Comparison of the effects of losartan and enalapril on clinical status and exercise performance in patients with moderate or severe chronic heart failure. J Am Coll

Cardiol 26:438-445, 1995.

362. Eberhardt RT, Kevak RM, Kang PM, Frishman WH. Ang II receptor blockade: An innovative approach to cardiovascular pharmacotherapy. J Clin Pharmacol 33:1023-1038, 1993.

363. van Wijngaarden J, Pinto YM, van Gilst WH, de Graeff PA, de Langen CDJ, Wessling H. Concerting enzyme inhibition after experimental myocardial infarction in rats: Comparative study between spirapril and zofenopril. Cardiovasc Res 25:936-942, 1991.

364. van Wijngaarden J, Monninlk SHJ, Bartels H, van Gilst WH, de Langen CDJ, Wessling H. Captopril modifies the response of infarcted rat hearts to isoprenaline stimulation. J Cardiovasc Pharmacol 19:741-747, 1992.

365. Sweet CS, Emmert SE, Stabilito II, Ribeiro LGT. Increased survival in rats with congestive heart failure treated with enalapril. J Cardiovasc Pharmacol 10:636-642, 1987.

366. Yamada Y, Endo M, Kohno M, Otsuka M, Takaiti O. Metabolic fate of the new angiotensin-converting enzyme inhibitor imidapril in animals. 1st communication: Absorption, pharmacokinetics and excretion in rats and dogs. Arzneim-Forsch/Drug Res 42:457-465, 1992.

367. Yamada Y, Endo M, Kohno M, Otsuka M, Takaiti O. Metabolic fate of the new angiotensin-converting enzyme inhibitor imidapril in animals. 2nd communication: Tissue distribution and whole-body autoradiography of imidapril in rats. Arzneim-

Forsch/Drug Res 42:466-474, 1992.

368. Yamada Y, Endo M, Kohno M, Otsuka M, Suzuki T, Takaiti O. Metabolic fate of the new angiotensin-converting enzyme inhibitor imidapril in animals. 3rd communication: Tissue accumulation after consecutive oral administration of [N-methyl-^{14}C]-imidapril in rats. Arzneim-Forsch/Drug Res 42:475-482, 1992.

369. Yamada Y, Endo M, Kohno M, Otsuka M, Takaiti O. Metabolic fate of the new angiotensin-converting enzyme inhibitor imidapril in animals. 5th communication: Isolation and identification of metabolites of imidapril in rats, dogs, and monkeys. Arzneim-Forsch/Drug Res 42:490-498, 1992.

370. Hirata Y, Hayakawa H, Kakoki M, Toji A, Suzuki E, Kimura K, Goto A, Kikuchi K, Nagano T, Hirobe M, Omata M. Nitric oxide release from kidneys of hypertensive rats treated with imidapril. Hypertension 27:672-678, 1996.

371. Ogiku N, Sumikawa H, Minamide S, Ishida R. Influence of imidapril on abnormal biochemical parameters in salt-loaded stroke-prone spontaneously hypertensive rats (SHRSP). Jap J Pharmacol 61:69-73, 1993.

372. Ogiku N, Sumikawa H, Hashimoto Y, Ishida R. Prophylactic effect of imidapril on stroke in stroke-prone spontaneously hypertensive rats. Stroke 24:245-252, 1993.

373. Fujiwara T, Yuasa H, Ogiku N, Kawai Y. Histopathological investigation on salt-loaded stroke-prone spontaneously hypertensive rats, whose biochemical parameters of renal dysfunction were ameliorates by administration of imidapril. Jap J Pharmacol 66:231-240, 1994.

374. Takahashi M, Fritz-Zieroth B, Ohta Y, Chikugo T. Therapeutic effects of imidapril on cerebral lesions observed by magnetic resonance imaging in malignant stroke-prone spontaneously hypertensive rats. J Hypertension 12:761-768, 1994.

375. Saruta T, Omae T, Kuramochi M, Limura O, Yoshinage K, Abe K, Ishii M, Watanabe T, Takeda T, Ito K, Kokubu T, Fujishima M, Arakawa K, Nakajima M. Imidapril hydrochloride in essential hypertension: A double-blind comparative study using enalapril maleate as a control. J Hypertension 13 (suppl. 13):S23-S30, 1995.

376. Ogiku N, Sumikawa H, Nishimura T, Narita H, Ishida R. Reduction of the mortality rate by imidapril in a small coronary artery disease model, (NEW x BXSB) F1 male mice. Jpn J Pharmacol 64:129-133, 1994.

377. Narta H, Kaburaki M, Doi H, Ogiku N, Yabana H, Kurosawa H, Ohmachi Y. Prolonging action of imidapril on the lifespan expectancy of cardiomyopathy hamsters. J Cardiovasc Pharmacol 27:861-871, 1996.

378. Vandenburg MJ, Mackay EM, Dews I, Pullan T, Brugier S. Dose finding studies with imidapril - a new ACE inhibitor. Br J Pharmacol 37:265-272, 1994.

379. Shao Q, Takeda N, Temsah R, Dhalla KS, Dhalla NS. Prevention of hemodynamic changes due to myocardial infarction by early treatment of rats with imidapril. Cardiovasc Pathobiol 1:180-186, 1996.

380. Pinto YM, van Veldhuisen DJ, Tjon-Ka-Jie RT. Dose-finding study of imidapril, a novel angiotensin converting enzyme inhibitor, in patients with stable chronic heart failure. Eur J Pharmacol 50:265-268, 1996.

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

382. Xu YJ, Panagia V, Shao Q, Wang X, Dhalla NS. Phosphatidic acid increases intracellular free Ca^{2+} and cardiac contractile force. Am J Physiol 271: H651-H659, 1996.

383. Xu YJ, Shao Q, Dhalla NS. Fura-2 fluorescent technique for the assessment of Ca^{2+} homeostasis in cardiomyocytes. Mol Cell Biochem, in press, 1997.

384. Grynkiewicz G, Poenie M, Tsien RY. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. J Biol Chem 260:3440-3450, 1985.

385. Bjornsson OG, Monck JR, Williamson JR. Identification of P2Y purinoceptors associated with voltage-activated cation channels in cardiac ventricular myocytes of the rat. Eur J Biochem 186:395-404, 1989.

386. Friel DD, Bean BP. Two ATP-activated conductances in bullfrog atrial cells. J Gen Physiol 91:1-27, 1988.

387. Pitts BJR. Stoichiometry of sodium-calcium exchange in cardiac sarcolemmal vesicles. J Biol Chem 254:6232-6235, 1979.

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

389. Taussky H, Shorr E. A microcaloric method for the estimation of inorganic phosphorous. J Biol Chem 202:678-685, 1953.

390. Ganguly PK, Pierce GN, Dhalla KS, Dhalla NS. Defective sarcoplasmic reticular

calcium transport in diabetic cardiomyopathy. Am J Physiol 244:E528-E535, 1983.

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

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

393. Zucchi R, Ronca-Testoni S, Yu G, Galbani P, Ronca G, Mariani M. Effect of ischemia and reperfusion on cardiac ryanodine receptors — sarcoplasmic reticulum Ca^{2+} channels. Circ Res 74:271-280, 1994.

394. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature Lond 227:680-685, 1970.

395. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Analytical Biochemistry 162:156-159, 1987.

396. Dubyak GR, Elmoatassim C. Signal transduction via P_2 purinergic receptors for extracellular ATP and other nucleotides. Am J Physiol 265:C577-C606, 1993.

397. Von Kugelgen I, Starke K. Noradrenaline-ATP co-transmission in the sympathetic nervous system. Trends Pharmacol Sci 12:319-324, 1991.

398. Soltoff SP, McMilliam MK, Talamo BR. ATP activates a cation-permeable pathway in rat parotid acinar cells. Am J Physiol 262:C934-C940, 1992.

399. McMillian MK, Soltoff SP, Cantley LC, Rudel RA, Talamo BR. Two distinct

cytosolic calcium responses to extracellular ATP in rat parotid acinar cells. Br J Pharmacol 108:453-461, 1993.

400. Cowen DS, Baker B, Dubyak GR. Pertussis toxin produces differential inhibitory effects on basal, P_2 purinergic, and chemotactic peptide-stimulated inositol phospholipid breakdown in HL-60 cells and HL-60 cell membranes. J Biol Chem 265:16181-16189, 1990.

401. Davidson JS, Wakefield IK, Sohnius U, Van der Merwe PA, Millar RP. A novel extracellular nucleotide receptor coupled to phosphoinositide-C in pituitary cells. Endocrinology 126:80-87, 1990.

402. De Young MB, Scarpa A. ATP-receptor induced Ca^{2+} transient in cardiac myocytes. Sources of mobilized Ca^{2+}. Am J Physiol 257:C750-C758, 1989.

403. Christie A, Sharma VK, Sheu SS. Mechanism of extracellular ATP-induced increase of cytosolic Ca^{2+} concentration in isolated rat ventricular myocytes. J Physiol 445:369-388, 1992.

404. Nijjar MS, Hart LL, Panagia V, Dhalla NS. Characterization of ATP-induced elevation in intracellular Ca^{2+} in rat cardiomyocytes. Cardiovasc Pathobiol 1:152-159, 1996.

405. Sethi R, Dhalla KS, Beamish RE, Dhalla NS. Differential changes in left and right ventricular adenylyl cyclase activities in congestive heart failure. Am J Physiol 272:H884-H893, 1997.

406. Sethi R, Dhalla NS. Inotropic responses to isoproterenol in congestive heart failure

subsequent to myocardial infarction in rats. J Cardiac Failure 1:391-399, 1995.

407. Basu S, Sinha SK, Shao Q, Ganguly PK, Dhalla NS. Neuropeptide Y modulation of sympathetic activity in myocardial infarction. J Am Coll Cardiol 27:1796-1803, 1996.

408. Ganguly PK, Dhalla KS, Shao Q, Beamish RE, Dhalla NS. Differential changes in sympathetic activity in left and right ventricles in congestive heart failure after myocardial infarction. Am Heart J 133:340-345, 1997.

409. Cheung JY, Musch TI, Misawa H, Semanchik A, Elensky M, Yelamarty RV, Moore RL. Impaired cardiac function in rats with healed myocardial infarction: cellular vs. myocardial mechanisms. Am J Physiol 266:C29-C36, 1994.

410. Capasso JM, Li P, Anversa P. Cytosolic calcium transients in myocytes isolated from rats with ischemic heart failure. Am J Physiol 265:H1953-H1964, 1993.

411. Book CB, Moore RL, Semanchik A, Ng YC. Cardiac hypertrophy alters expression of Na^+, K^+ ATPase subunit isoforms at mRNA and protein levels in rat myocardium. J Mol Cell Cardiol 26:591-600, 1994.

412. Book CB, Wilson RP, Ng YC. Cardiac hypertrophy in the ferret increases expression of the Na^+-K^+ ATPase α_1- but not α_3-isoform. Am J Physiol 266:H1221-H1227, 1994.

413. Allen PD, Schmidt TA, Marsh JD, Kjeldsen K. Na, K-ATPase expression in normal and failing human left ventricle. Basic Res Cardiol 87 (Suppl 1):87-94, 1992.

414. Kent R, Rozich JD, McCollan PL, McDermott DE, Thacker UF, Menick DR,

McDermott PJ, Cooper G. Rapid expression of the Na^+-Ca^{2+} exchanger in response to cardiac pressure overload. Am J Physiol 265:H1024-H1029.

415. Zhang XQ, Tiliotson DL, Moore BL, Zelis RZ, Cheung JY. Na^+/Ca^{2+} exchange currents and SR Ca^{2+} contents in postinfarction myocytes. Am J Physiol 271:C1800-C1807, 1996.

416. Howl LC, Whalley DW, Doohan MM, Rasmussen HH. Angiotensin-converting enzyme inhibition, intracellular Na^+, and Na^+-K^+ pumping in cardiac myocytes. Am J Physiol 268:C366-C375, 1995.

417. Ottlecz A, Besaoula T. Captopril ameliorates the decreased Na^+, K^+-ATPase activity in the retina of streptozotocin-induced diabetic rats. Invest Ophthalmol Vis Sci 37:1633-1641, 1996.

418. 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 failure and nonfailure human myocardium. Circ Res 75:434-442, 1994.