PHOSPHOINOSITIDE-PHOSPHOLIPASE C IN NORMAL AND FAILING HEARTS TREATED WITH IMIDAPRIL

BY

SHALINI SHATADAL

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

MASTER OF SCIENCE IN PHYSIOLOGY

Department of Physiology
Faculty of Medicine
University of Manitoba

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LIST OF ABBREVIATIONS

ACE.....Angiotensin converting enzyme

AMI.....Acute myocardial infarction

Ang-I.....Angiotensin-I

Ang-II.....Angiotensin-II

ANP.....Atrial natriuretic peptide

cDNA.....Complimentary deoxyribonucleic acid

CHF.....Congestive heart failure

DAG.....*sn* 1,2-Diacylglycerol

G-protein.....Guanine nucleotide binding protein

GDP.....Guanine diphosphate

GTP.....Guanine triphosphate

Ins(1)P.....Inositol mono-1phosphate

$Ins(1,3,4,5)P_4$.....Inositol tetrakis-1,3,4,5phosphate

$Ins(1,4)P_2$.....Inositol bis1,4phosphate

$Ins(1,4,5)P_3$.....Inositol tris-1,4,5phosphate

LV.....Left ventricle

mRNA.....Messenger ribonucleic acid

Na^+.....Sodium

NE.....Norepinephrine

PA.....Phosphatidic acid

PC.....Phosphatidylcholine

PKC.....Protein kinase C

PLC.....Phospholipase C

PLD.....Phospholipase D

PRA.....Plasma renin activity

proANP.....pro-Atrial natriuretic peptide

PtdIns(4,5)P_2.....Phosphatidylinositol 4,5-bisphosphate

PtdIns.....Phosphoinositide

PtIns(4)P.....Phosphatidylinositol 4-phosphate

PtdIns kinase.....Phosphoinositide kinase

PtIns(4)P.kinase.....Phosphatidylinositol 4-phosphate kinase

RAS.....Renin angiotensin system

SDS-PAGE.....Sodium dodoecyl sulphate - polyacrylamide electrophoresis

SL.....Sarcolemma

SR.....Sarcoplasmic reticulum

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ABSTRACT

Congestive heart failure subsequent to myocardial infarction (MI) is a common clinical syndrome usually with a grave prognosis. It is a complicated interplay between mechanical and humoral factors attempting to attain circulatory homeostasis after the myocardium is damaged. Loss of myocardium i.e. development of myocardial infarction and subsequent scar formation, produces extra work load on the remaining non-infarcted myocardium. The heart responds by an increase in muscle mass due to increased cell volume in the individual cardiac myocytes, termed the hypertrophic process. In addition, neurohormonal mechanisms such as the renin angiotensin system (RAS), is also activated to restore circulatory homeostasis. The compensatory mechanisms activated are helpful at first but generally have a deleterious effect over a prolonged period. Therefore, if this condition persists, CHF develops.

Ang-II, a potent vasoconstrictor, is the final product of the RAS. Ang-II, is formed from angiotensin-I (Ang-I) under the action of angiotensin converting enzyme (ACE). Ang-II couples via a G-protein to phospholipase C which preferentially hydrolyses phosphatidylinositolbis(4,5)phosphate to form inositoltris(1,4,5) phosphate and *sn-1,2* diacylglycerol (DAG).

Inositoltris(1,4,5)phosphate mobilizes intracellular stores of calcium in the sarcoplasmic reticulum and DAG activates protein kinase C which, in turn, phosphorylates a number of intracellular proteins.

ACE-inhibitors are effective in slowing down the progression of congestive heart failure after myocardial infarction. The present study was undertaken to test the hypothesis that phosphoinositide specific phospholipase C (PI-PLC) activity is altered (depressed) in congestive heart failure (CHF) and that such changes can be corrected by ACE-inhibitor treatment.

PI-PLC activity was measured in the sarcolemma and cytosol isolated from left ventricles of experimentally induced CHF animals at post infarction periods of 1, 2, 4, and 8 weeks. The ACE-Inhibitor Imidapril, a new experimental drug, was orally administered (1mg/ kg/day of the body weight) to animals. Drug treatment was initiated at the beginning of the fifth week and continued until the end of eighth week. In the sarcolemma, the phosphoinositide phospholipase C (PI-PLC)activity at 2, 4, and 8 weeks after myocardial infarction were significantly depressed by 17, 19, 23% ($P<0.05$) respectively. At one week , however, no significant ($P>0.15$) difference was observed in PI-PLC activity in sarcolemma. Upon Imidapril treatment, sarcolemmal PI-PLC activity exhibited a partial but significant recovery in the CHF group at 8 weeks as compared to sham control. In cytosolic fraction, no

significant changes in PI-PLC activity were observed at 1, 2, 4, and 8 week animals. Similarly, Imidapril treated animals showed no change in PI-PLC activity in the cytosol. The viable left ventricle (LV)/ body weight ratio was also calculated in 8 week animals. A significant ($P<0.05$) increase in the LV weight /body weight ratio was observed, indicating the presence of left ventricular hypertrophy in the hearts of 8 week post infarcted animals. Whereas on Imidapril treatment LV weight /body weight ratio was significantly decreased ($P<0.05$). The present study indicates that PI-PLC activity in CHF was depressed which was partially corrected by imidapril treatment. Furthermore, the data show these changes in PI-PLC activity are sarcolemmal specific.

I. INTRODUCTION

Congestive heart failure (CHF) is a complex response to compromised cardiac output characterized by inadequate oxygen delivery for normal tissue metabolism. CHF can be characterized by easy fatigability, diminished exercise tolerance, dyspnea and peripheral edema. The pathological processes that contribute in the initiation and progression of CHF are: loss of cardiac muscle, pressure overload, volume overload, decreased contractility and restricted filling. Crucial biochemical mechanisms including excitation-contraction coupling and intracellular cardiac calcium metabolism become defective in CHF.

Loss of coronary flow leads to myocardial infarction as well as a net loss of the contractile myocardium. This in turn results in an increase in wall stress on the non-infarcted myocardium with attendant myocardial hypertrophy. The RAS, sympathetic nervous system and atrial natriuretic peptide (ANP), are activated within a few hours after coronary ligation as a consequence of reduced cardiac output. Neurohormonal activation may also be a key factor in the progressive deterioration of left ventricular function and contribute to the natural history of the syndrome (Cohn, 1990).

The RAS is integrally involved in cardiovascular homeostasis. Ang -II, a potent vasoconstrictor, is the principal mediator of RAS. Ang-II stimulates a

trimeric G protein, which in turn activates a phosphoinositide specific phospholipase C (PLC), a phosphodiesterase enzyme. The PLC activation causes hydrolysis of PtdIns(4,5)P_2 yielding two second messengers $InsP_3$ and DAG. $InsP_3$ mobilizes intracellular stores of calcium in the sarcoplasmic reticulum. DAG activates number of isoenzymes of protein kinase C (PKC). $InsP_3$, is further phosphorylated to $InsP_4$ and both $InsP_3$ and $InsP_4$ may have an important role in controlling intracellular cardiac calcium homeostasis. The PI-pathway plays a part in inducing cardiac hypertrophy (Shoki *et al.*, 1992). Progressive heart diseases are treated with ACE-inhibitors, and the clinical relevance of these drugs is indicated by blocking the conversion of Ang-II from Ang-I which occurs in several pathological conditions (Baker *et al.*, 1992). ACE-inhibitor therapy has been shown to result in an improvement in the quality of life, an increase in exercise capacity and a reduction in admission of patients to the hospital. ACE-inhibitors reduce mortality not only when used for CHF, but also after an acute myocardial infarction (AMI). The efficacy of ACE-inhibitor treatment for patients in mild to moderate stages of heart failure has encouraged physicians to recommend these drugs as first line therapy.

Imidapril, a recently developed experimental ACE-inhibitor agent, belongs to the class containing a carboxyl group, as do all drugs of this type. Imidapril inhibits the conversion of Ang-I to Ang-II. Upon oral administration, Imidapril is absorbed in the gastrointestinal tract and subsequently eliminated

via the kidneys. It has fewer side effects than Captopril and is slightly more potent than Enalapril (Vandenburg *et al.*, 1994). It improves hemodynamic function and extends the life of rats with CHF subsequent to MI (Yabana *et al.* 1992).

The present study had two specific objectives: (1) to examine phosphoinositide specific Phospholipase C activity in sarcolemmal and cytosolic fractions in experimentally induced heart failure in rats and (2) to determine the changes in the phosphoinositide specific Phospholipase C activity in these subcellular compartments upon treatment with the Imidapril.

The research was designed to test the hypothesis that, phosphoinositide specific Phospholipase C activity may be downregulated during CHF and improved prognosis with ACE-Inhibitor therapy may be associated with the recovery of PI-PLC activity.

II. BACKGROUND

It is estimated that heart failure afflicts more than three million people in North America. Approximately 500,000 people develop congestive heart failure every year. The patients suffering from this clinical syndrome have a high mortality rate from the time of diagnosis. For men the mortality is about 60% and for women it is about 45%. In this regard, the most prevalent cause of this pathologic state is the presence of coronary artery disease, while systemic hypertension and valvular disease are also important. However, the most common pathologic state is left ventricular failure after the occurence of left ventricular myocardial infarction.

The medical treatment of CHF has undergone tremendous advancement in the past two decades. The new and improved studies on the pathophysiology of CHF has expanded the paradigm of understanding of this disease. Treatment has gone from a pathophysiological approach to an evidence guided approach. Further, medications which improve functional capacity and reduce morbidity and mortality are now available.

Ang-II is produced by RAS, it is a potent vasoconstrictor which is formed from its precursor Ang-I, Ang-I by activity of the angiotensin converting enzyme (ACE). ACE activity is present in the circulation, as well as locally, appearing in the lungs and in other tissues including the

myocardium and coronary arteries. Ang-II exerts a number of direct effects on the heart via a G-protein. It causes hydrolysis of phosphatidylinositol and activates protein kinase C (PKC) through the formation of diacylglycerol (DAG) (Gavras, 1994). The positive inotropic, mitogenic and arrhythmogenic actions of Ang-II are now well documented. Withdrawal of Ang-II would cause hemodynamic improvement (preload, enhanced coronary blood flow) and metabolic consequences (diminish myocardial oxygen demand) during treatment of CHF.

Several factors contribute to CHF, however, the most important is the loss of cardiac muscle due to myocardial infarction and subsequent scar formation (Paul, 1995). As the disease progresses there is a depression in the hearts pumping ability and ventricular reserve. The loss of heart muscle during infarction produces increased workload on remaining viable myocardium. In addition, the heart responds by an increase in muscle mass known as hypertrophy, a compensatory mechanism to maintain adequate perfusion of vital organs (Dhalla *et al,* 1993). However, if this condition persists for a prolonged period, the onset of CHF becomes imminent.

In response to reduced cardiac output, neurohormonal mechanisms are activated to restore circulatory homeostasis. In CHF, there is increase in circulating catecholamine levels, sympathetic tone and ANP, and an excessive activation of RAS . Together, these systems, exacerbate the declining heart

function in patients. These compensatory mechanisms, helpful at the initial stages, become deleterious to the proper functioning of the cardiovascular system over a prolonged period. Recently, it has been shown that the sequence of events associated with the activation of neurohormonal system, and in particular the RAS may be abrogated when appropriate treatment is administered given to patients.

Progressive heart disease can be attenuated by ACE-inhibitor therapy. ACE-inhibitor therapy is beneficial when administered for a month beginning the week following myocardial infarction. These ACE-inhibitor drugs demonstrate an improvement in symptoms and exercise tolerance. They also reduce morbidity and mortality (Kleber *et al.*, 1991). In addition, ACE-inhibitor efficacy in patients with mild to moderate heart failure has encouraged physicians to recommend that these drugs be administered as the agent of first line therapy for the treatment of CHF (Gavras, 1990, 1991) . However, both experimental and clinical reports and data have revealed that coronary occlusion results in activation of RAS. These studies lead to the speculation that activation of the RAS may exacerbate myocardial ischaemic injury and thus in this case there is a possibility that ACE-inhibitors act as a cardioprotective agents (Dzau, 1990; Paul, 1995). In addition there is an alteration in calcium homeostasis such that cardiac contractility is modified in heart failure. Thus, the possibility arises that factors which can increase

calcium levels may also increase contractile force. PLC is one such factor that generates the second messenger inositol tris phosphate which mobilizes intracellular calcium stores. Therefore , PLC becomes important as it plays major role in calcium homeostasis in heart failure.

1. PHOSPHOINOSITIDE PATHWAY

Gut and Huggins (1966) were the first to describe the α_1-adrenergic and muscarinic stimulation of ^{32}P-Na^+ orthophosphate incorporation into the total polyphospho-phosphatidylinositol fraction of the membrane *in vivo*. Authors termed this response as the "phosphoinositide effect". The PtIns turnover starts with the phosphorylation of PtIns to PtIns(4)P by PtIns kinase, which in turn is phosphorylated to PtIns(4,5)P_2 by PtIns(4,5)P_2 kinase; the number refers to the position of the phosphate on the inositol ring (Wolf *et al*, 1990; Meldrum *et al*, 1991). Collectively, these three phospholipids, PtIns, PtIns(4)P and PtIns(4,5)P_2 constitute approximately 8% of the total phospholipids (Hawthorne, 1992; Meldrum *et al*, 1991).

Several receptors such as α1 adrenergic, angiotensin II, muscarinic, endothelin-1, thrombin adenosine nucleotide and opioid peptide receptors are coupled via a G-protein to phosphoinositide specific PLC which hydrolyses PtIns(4,5)P_2 (the preferred substrate of phosphoinositide specific PLC

enzyme) in the myocardium (Lamers *et al*, 1993; Terzic *et al*, 1993). The hydrolysis of PtIns(4,5)P_2 produces two second messengers, Ins(1,4,5)P_3 and DAG (Fain, 1990; Lamers *et al*, 1993; Meij *et al*, 1994). The second messenger Ins(1,4,5)P_3 is a water soluble molecule which triggers the mobilization of calcium into the cytoplasm of stimulated cells. Ins(1,4,5)P_3 is metabolized by two routes. A part is dephosphorylated to Ins(1,4)P_2, causing its immediate inactivation and the remainder is phosphorylated to Ins(1,3,4,5)P_4 (Berridge and Irvine, 1989; Lamers *et al*, 1992; Lamers, 1993). The second messenger DAG activates several isoenzymes of PKC (Nishizuka, 1988; Lamers, 1992, 1993). Figure 1. shows a schematic representation of the PI pathway.

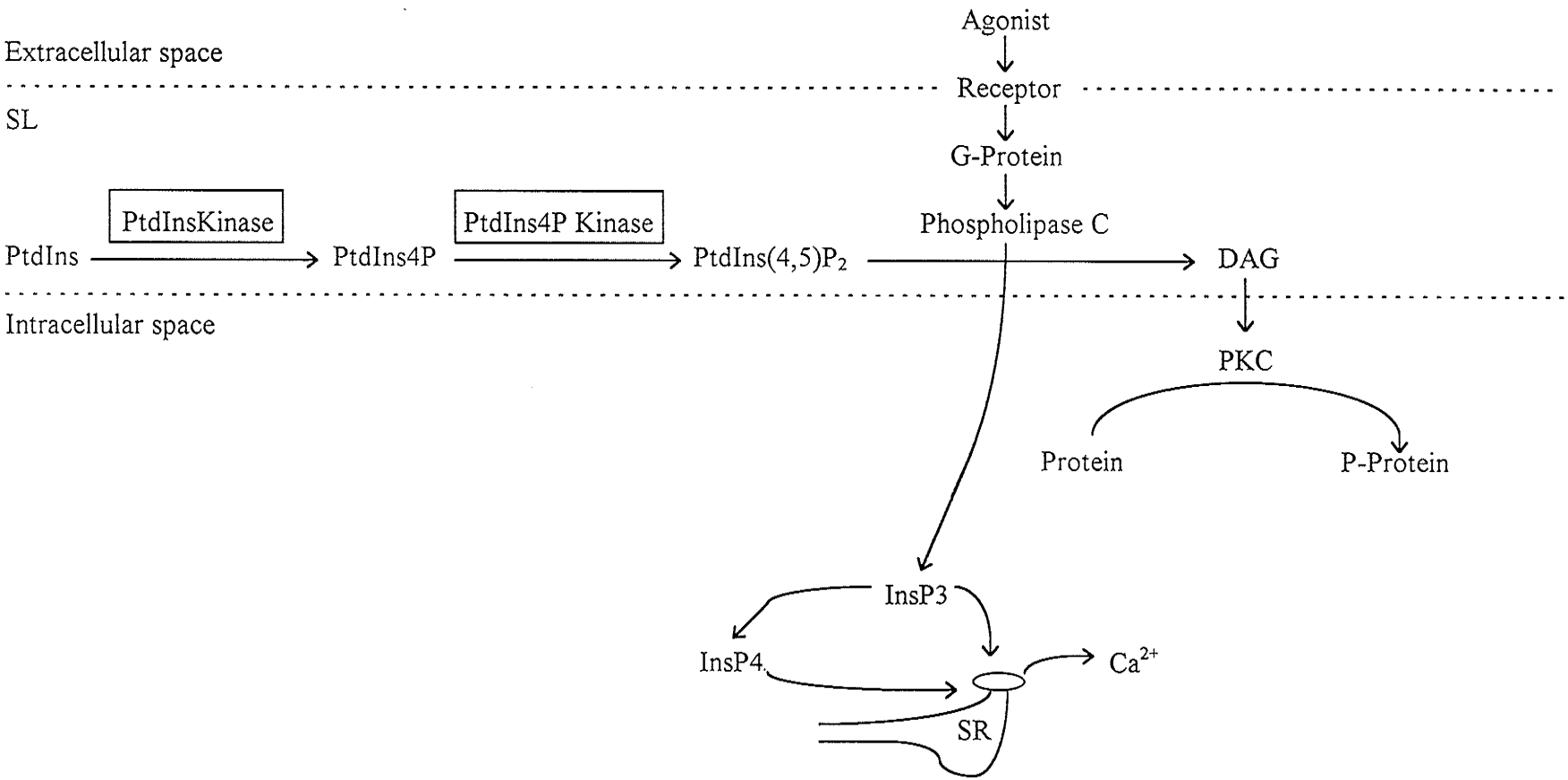


Figure 1. A schematic representation of the phosphoinositide pathway. Abbreviations : PtdIns = Phosphoinositide, PtdInsKinase = Phosphoinositide kinase, PtdIns4P = Phosphatidylinositol 4 phosphate, PtdIns4P kinase =Phosphatidylinositol 4phosphate kinase, PtdIns(4,5)P_2 =Phosphatidylinositol bis(4,5) phospahte,DAG = *sn-1,2* diacylglycerol, PKC =protein kinase C, $InsP_3$ = Inositol tris (1,4,5) phosphate, $InsP_4$ = Inositol tetrakis(1,3,4,5) phosphate, SR = sarcoplasmic reticulum, SL = sarcolemma.

2. REGULATION OF THE PHOSPHOINOSITIDE PATHWAY

2.1. G-Proteins

The G-protein is a member of a large family of GTP binding proteins that act as a signal transducer across cell membranes. The paramount role of G-proteins in signal transduction has made it a subject of intence interest during the past decade (Gilman, 1987, Neer and Clapham, 1988; Johnson and Dhanesekaran, 1989; Birnbaumes, 1990; Holmer and Homcy, 1991; Fleming *et al*, 1992; Thomas, 1993). Heteromeric G-proteins are composed of three different polypeptide chains, namely α, β, and γ. It is the α-chain which binds the GTP in the active state of the G-protein. The β- and γ-chains form a tight $\beta\gamma$ complex which binds the protein to the cytoplasmic face of the sarcolemma. Based on the amino acid homology, twenty isoforms of α subunit have been identified and grouped into five different classes. These five classes of α subunits (corresponding G-protein) are α_s (G_α), α_i (G_i), α_q (G_q), α_o (G_o) and α_{12} (G_{12}). Different α-subunits interact with different effectors. Four isoforms of β and six isoforms of γ subunits have also been reported (De Jonge *et al*, 1995).

A very large number of membrane bound receptors that activate various α-subunits of G-proteins after binding with a signalling ligand are called G-

protein linked receptors. An array of diverse agonists including neurotransmitters and hormones such as serotonin and epinephrine, large proteohormones, light and olfactory stimuli may transfer the signal to a cell via a G-protein like receptor (Lohse, 1995). All these receptors share a common basic structure that can be characterized by an extracellular N-terminus, seven membrane-spanning α helices and a cytoplasmic C-terminus of variable length (DeJonge *et al*, 1995).

The signalling cascade begins when a hormone or neurotransmitter binds to a G-protein linked receptor which interacts with and activates a heterotrimeric G-protein. In the activated protein, GTP is exchanged for GDP. The α-GTP complex dissociates from the $\beta\gamma$ complex to activate the effector molecule. The hydrolysis of GTP into GDP by GTPase activity of the G-protein causes self inactivation of the α subunit and the formation of heterotrimer again (Thomas, 1993).

The most important examples of signal transmitted by the α subunit are stimulation and inhibition of adenylyl cyclase by α_s and α_i respectively and the stimulation of PLC by α_q. The $\beta\gamma$ complex can also stimulate or inhibit a few isoforms of adenylyl cyclase and it can activate PLC but very mildly.

2.2. Phosphoinositide Specific Phospholipase C

The Phosphoinositide specific PLC belongs to a class of phosphodiesterase that cleaves inositol phospholipid to DAG and Ins(1,4,5)P_3 in most mammalian cells, plants and micro-organisms (Shukla, 1982; Rhee *et al*, 1984). The first PLC enzyme was purified to homogeneity from rat liver, when subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (Takenawa and Nagai, 1981; Rhee *et al*, 1989). Subsequently, several distinct types of PLC isoenzymes have been purified from mammalian tissues (Rhee *et al*, 1989; Rhee and Choi, 1992). PLC isoenzymes were classified on the basis of their deduced amino acid sequence from their cloned cDNA and their sequence homology. Comparison of amino acid sequence indicated that PLC isoenzymes could be divided into three types, PLC-β, PLC-γ and PLC-δ. Within each family of PLC isoenzymes there are several subtypes. The subtypes are designated by adding Arabic numerals after the Greek letters as in PLC-β_1, PLC-β_2 (Cockcroft and Thomas, 1992). Three PLC-β, two PLC-γ and two PLC-δ isoenzymes have been reported so far. Only PLC-β isoenzymes have been found to be stimulated by G_q family of G-proteins (De Jonge *et al*, 1995). PLC-β is the most active, followed by PLC-β_3, while PLC-β_2 exhibits weak activity. Such differences in activity suggest different interaction of α-

subunit of G-protein with different PLC-β isoenzymes. In heart, the most abundant PLC isoenzyme is PLC-γ followed by PLC-β and PLC-δ (Neer and Clapham, 1992). Among the subtypes of PLC-β only PLC-β_1 and PLC-β_3 are present in the myocardium.

2.3. Protein Kinase C

PKC plays a role in the action of a number of hormones and growth factors in a variety of tissues. The major activator of PKC is DAG which is formed after the stimulation of membrane phospholipid hydrolysis by agonists (Speechly-Dick *et al.*, 1994). DAG is produced by phospholipid hydrolysis of $PtdInsP_2$ and serves to activate PKC. DAG may also be derived form the hydrolysis of PC by a specific PLC or by PLD. PKC phosphorylates the PLC enzyme and the G-proteins of other signal transduction pathways (Huang, 1989; Rhee et al. 1989). Rhee et al. (1989) reported that PLC β_1, γ_1, δ_1, is phosphorylated *in vitro* without attenuation of PLC activity. The PKC has been shown to phosphorylate at the serine residue of the PLC β_1 that mediates the PLC and G protein(Liscovitch, 1992). The PKC isoenzymes are stimulated by DAG with varying potency in assays containing different phospholipids

(Huang, 1989). In general, PKC-I is more sensitive to stimulation by DAG or phorbol esters than PKC-II and PKC-III (Huang, 1989).

In addition, PKC is recognized to be a group of enzymes encoded by a family of genes, which is further divided in two groups, the cPKC and the nPKC (Ono *et al,* 1988; Bacher *et al.,* 1991; Osada *et al.,* 1990, 1992; Ohno *et al,* 1988). The cPKC groups consisted of four isoenzymes, PKC-α, PKC-β_1, PKC-β_2 and PKC-γ. The nPKC group contains nPKC-δ, nPKC-ε, nPKC-ξ, nPKC-η and nPKC-σ. PKC inhibitors such as calphostin c, gremophorel and AMG (1-0 alkyl 2-0 methyl glycerol) act by interfering with the interaction of DAG or phorbol with PKC (Ono *et al,* 1989; Selbie *et al.,* 1993).

Tumor promoting phorbol esters that activate the PKC family have been used extenstively to demonstrate the involvement of PKC in the regulation of many cellular processes. It shows an effect on contractility and protein synthesis in the adult heart. Phorbol esters have been implicated in the induction of hypertrophy in neonatal rat hearts (Bogoyevitch *et al.*, 1993).

2.4. CROSS-TALK BETWEEN PLC AND PLD

The role of PKC in the signal transduction pathway is well established (Liscovitch, 1992). PKC is activated by DAG which, in turn, phosphorylates a number of proteins. DAG can be derived from the hydrolysis of

phosphoinositide and phosphatidylcholine (PC) by their specific phospholipases. The hydrolysis of PtdIns(4,5)P_2 by PtIns specific PLC was described in the PtIns-cycle section. Another potential source of DAG is the hydrolysis of phosphatidyl choline (PC) by phospholipase D (PLD), generating phosphatidic acid (PA) and choline (Exton, 1990; Liscovitch, 1992). PA is dephosphorylated to DAG by phosphomonoesterase. The PtIns(4,5)P_2 hydrolysis yields a relatively small amount of DAG and for a shorter period of time compared to PC hydrolysis. This suggests that the cellular content of PC is several folds higher than PtIns(4,5)P_2 (Exton, 1990). Further, it was demonstrated that PC hydrolysis leads to the production of DAG that is an effective activator of PKC *in vitro*. Also, activation of PKC by PC hydrolysis is likely to be more pronounced and for a longer duration than PtIns(4,5)P_2 breakdown (Exton,1990). This property may be an important factor in cellular control mechanisms. In addition, Qian *et al* (1991) showed that in certain cells PLD activity was enhanced by increasing the calcium concentration. It may be the consequence of inositol phosphate elevation by PLC action after receptor stimulation. As the mechanism of the signalling process unfolds, it may become clear that cross-talk between phospholipases is essential to accomplish some of their important function.

Panagia *et al* (1991) were the first group of investigators to demonstrate the presence of PLD in sarcolemma purified from rat myocardium. The

stimulation of isolated rat hearts by endothelin-1 produced both PC breakdown and elevation of inositoltris phosphate ($Ins(1,4,5)P_3$) levels (Prasad, 1991). Further evidence was reported by Pesin and Raben in 1989 after studying the molecular species of cellular DAG by α-thrombin stimulation of cultured fibroblasts. They showed that $PtIns(4,5)P_2$ contributed a significant proportion of the DAG which was generated within 15 seconds of the addition of α-thrombin, while PC induced DAG generation occured after five minutes to one hour.

The activation of PLD and PLC by the same calcium mobilizing hormone raises the possibility that both PLD and PLC enzymes might be regulated by the same G-protein. It is interesting to note that on one hand PKC promotes the interaction of the G-protein with PLD, enhancing PLD activation. On the other hand, PKC exerts a negative feedback action on G-protein, thus shutting off $PtIns(4,5)P_2$ hydrolysis (Exton, 1990; Liscovitch, 1991; 1994). Thus, if both PLC and PLD are regulated by the same G-protein, the cross-talk between the two enzymes, PLC and PLD, might be the mechanism by which cardiomyocytes can maintain increased levels of DAG for longer periods of time.

2.5. Other Modulatory Factors

There are several other modulatory factors which increase the phosphorylation of PLC in the phosphoinositide pathway. Tyrosine

phosphorylation of phosphoinositide specific Phospholipase C is implicated in the stimulation of PIP_2 hydrolysis (Morrison *et al.* 1990). They also described that the addition of epidermal growth factor (EGF) or platelet derived growth factor (PDGF) to cells leads to an increase in the amount of phosphotyrosine and phosphoserine on PLC γ-1. Also, there are multiple sites of tyrosine phosphorylation of PLC γ-1 *in vitro* and *in vivo* (Wahl *et al.* 1992). PLC γ-1 activity is also regulated by phosphatidic acid and lysophosphatidic acid, both of which may cause an increase in PIP_2 hydrolysis (Jones and Carpenter, 1993).

3. CONGESTIVE HEART FAILURE

With the progression of congestive heart failure, in addition to heart, many other organs such as kidney, lung, liver, skeletal muscle, blood vessels and brain become affected. In case of heart failure changes could be at subcellular, cellular, and organ level. In this regard, significant changes in the calcium metabolism and myosin isoenzymes in CHF have been reported.

3.1. Myosin isoenzymes

There are three myosin isoenzymes depending on the composition of the myosin. They are described as V_1, V_2 and V_3 and composed of α-α, α-β and β-

β subunits respectively (Schwartz *et al.*, 1981; Rupp *et al.*, 1986). Remodelling during CHF shifts the composition of the myosin isoenzyme. The V_1 myosin isoenzyme, which is present in normal hearts, shifts to the V_3 isoenzyme during CHF in viable myocardium. The decrease in ATPase activity slows the crossbridge cycle, which is also an important factor in the progression of CHF (Zimmer *et al.*, 1989).

3.2. Calcium Homeostasis

Calcium plays an important role in contractile performance in cardiac muscle. The release and re-uptake of calcium from intracellular stores is crucial for normal contraction and relaxation in the mammalian heart (Mansour and Morgan., 1995). Consequently, the abnormal handling of intracellular calcium has been identified as one of the major mechanisms leading to CHF (Rasmussen., 1986; Katz., 1990; Morgan., 1991). There are two major sites of calcium regulation, the sarcolemma and sarcoplasmic reticulum, which are monitored on the basis of action potential and calcium transient. Calcium is predominantly regulated by the troponin-tropomyosin complex (Mansour and Morgan., 1995). In addition, membrane alteration and enzyme activity changes result in a decrease in calcium uptake thereby causing

an increase in intracellular calcium stores, which also effects excitation-contraction coupling (Dhalla *et al.*, 1991).

Impairment in calcium handling is manifested by a marked increase in systolic and diastolic calcium concentrations associated with a marked decrease in force production (Kihara *et al.*, 1989.; Carrozza et al., 1992). Thus the abnormalities produced in the regulation of intracellular calcium appears to be initially a compensatory mechanism in cardiac hypertrophy, but ultimately these changes may contribute to heart failure (Morgan., 1991).

3.3. THE NEUROHORMONAL SYSTEM

Recently, several neurohormones have been implicated in the progression of congestive heart failure. The level of endocrine activation has been directly correlated with the degree of ventricular impairment and mortality (Pratt *et al*, 1995). The precise mechanism of each hormone causing ventricular dysfunction remains to be elucidated. Following is a brief account of some of the neurohormonal changes.

3.3a. The Renin Angiotensin System

A detailed review of the renin angiotensin (RAS) system in congestive heart failure has been described by Cody and Laragh (1988). The development of radioimmunoassay, techniques for protein isolation and sequencing and the

availability of synthetic peptides and pharmacologic inhibitors have made it easy to understand the co-relation between RAS and cardiovascular physiology.

The RAS was first noticed as a blood borne biochemical cascade whose final product is Ang-II. However, the complete RAS components have been now reported in many tissues including heart. The Ang-II is one of the most potent vasoconstrictors and it acts preferentially on arterial vessels. Briefly, renin is the first enzyme in the biochemical cascade of the RAS. The main source of renin is the juxtaglomerular cells of the afferent arteriole of the kidney, where it is synthesized and stored. Renin is an enzyme with high specificity for its substrate, angiotensinogen. Angiotensinogen is cleaved, synthesized and stored in the liver. Angiotensinogen is cleaved by renin to release physiologically inactive peptide Ang-I (Bouhnik *et al*, 1981). Ang-I is cleaved by ACE, a dipeptidyl peptidase to produce Ang-II. Ang-II, the physiologically active octapeptide, has multiple sites of action.

A schematic representation of the RAS is shown figure 2. Ang-II has a short plasma life and is degraded by several peptidases (Morton, 1993). Although ang-I is considered to be physiologically inactive, it is capable of stimulating catecholamine release from the adrenal medulla (Peach, 1974). Abnormal levels of Ang-II in plasma and tissues leads to an increase in total peripheral resistance causing increased blood pressure and afterload. It also

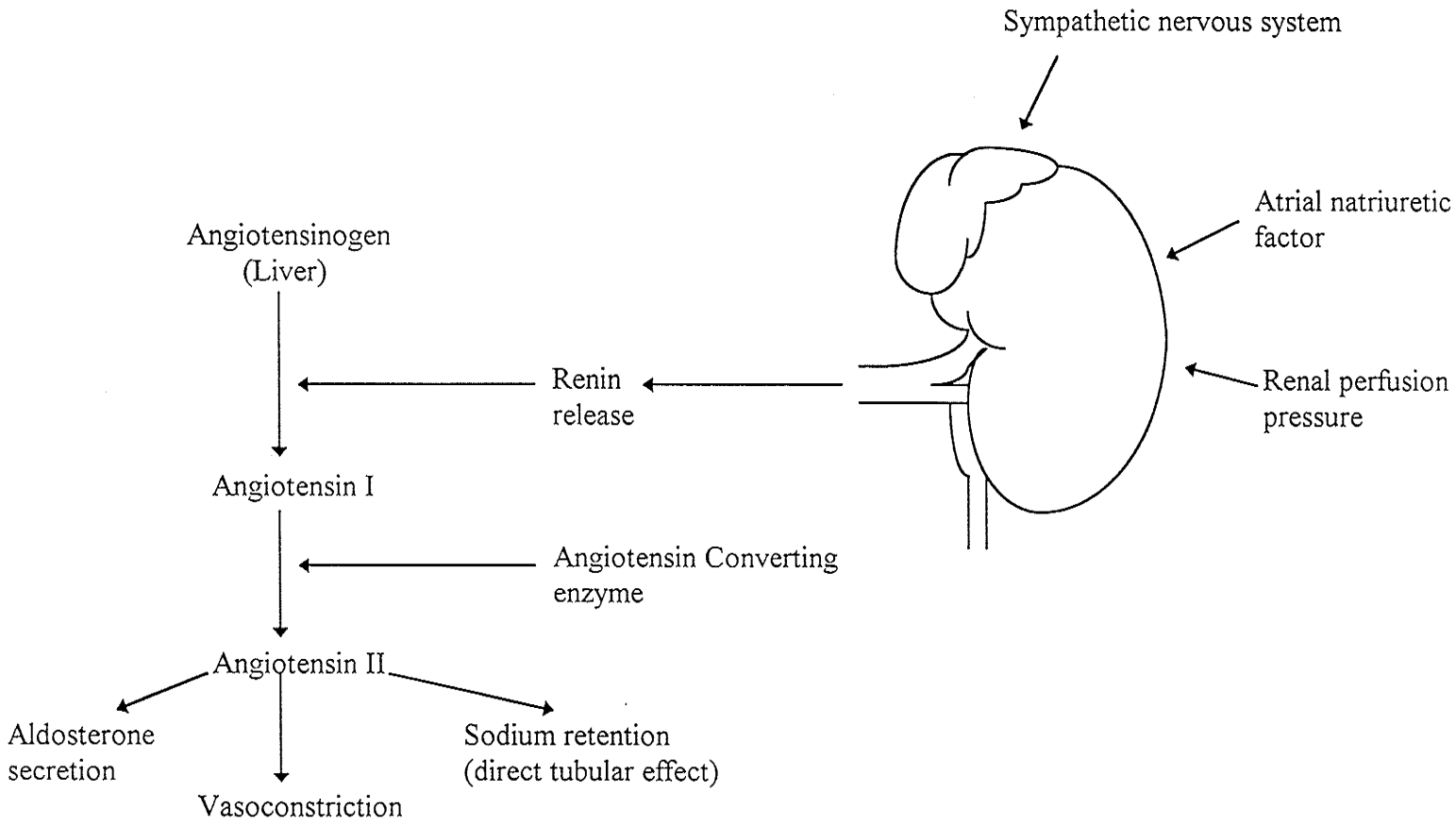


Figure 2. A schematic representation of Renin Angiotensin System.
(Compiled from Dzau and Pratt, 1986)

stimulates the adrenal cortex to release aldosterone which causes the kidneys to reabsorb sodium and water in exchange for potassium. This results in an increase in blood volume, blood pressure and preload. Ang-II also plays an important role in the growth and development of cells (Francis, 1990). Some data suggests that Ang-II acts on a membrane bound receptor to activate the $Ins(1,4,5)P_3$ and DAG pathways, leading to increased intracellular calcium and activation of PKC (Alexander *et al*, 1985; Nabika *et al*, 1985; Francis, 1990).

The existence of RAS in the heart was demonstrated by Baker *et al* (1992). The components of RAS, such as renin, angiotensinogen, ACE, Ang-I and Ang-II, have been identified in cardiac membranes (Kawaguchi and Kitabatake, 1995). The mRNA for angiotensinogen, prorenin and ACE have been found in cardiac membrane of rats (Nakashima *et al*, 1994). In addition, in 1992 activation of the RAS and the resultant release of Ang-II were implicated in the pathophysiology of cardiovascular disease (Dahlof *et al*, 1992).

3.3b. Atrial Natriuretic Peptide (ANP)

Jamieson and Palade (1964) described the presence of granules in the myocyte of human atrium. In 1981, DeBold et al showed that atrial extracts from rats caused considerable natriuresis. Shortly thereafter, it was demonstrated that similar atrial extracts produced vasodilation in vascular and non-vascular smooth muscle preparations (Currie et al, 1983; Laragh, 1985).

ANP is a cardiac hormone, which exists as a prohormone in the heart and is stored as a 126 amino acid peptide within atrial granules. ProANP is cleaved by a membrane protease in response to atrial stress. It is released into the circulation as a 28 amino acid C-terminal peptide and a 98 amino acid N-terminal peptide. The N-terminal peptide has a lower clearance rate and a longer half life compared to the C-terminal peptide. As a consequence, the N-terminal ANP circulates in higher concentration (Vesley *et al.*,1987;Winters *et al,* 1988; Pevahouse *et al,* 1989; Ngo *et al,* 1989; Martin *et al,* 1990). It is believed that ANP interacts with RAS to perform various functions. It opposes Ang-II mediated vasoconstriction of blood vessels, reduces renin secretion from the juxtaglomerular apparatus of the kidney, blocks angiotensin stimulated aldosterone secretion from the adrenal cortex and also opposes aldosterone induced renal sodium and water (Weber and Villarreal, 1993).

ANP has been identified as a diagnostic and prognostic marker for congestive heart failure (Francis,1990) and it may play a role in maintaining

the compensated asymptomatic state of left ventricular dysfunction. ANP levels is consistently elevated in early asymptomatic ventricular dysfunction, making it useful as a predictor for development of congestive heart failure. Elevated circulating levels of ANP are associated with worsening of congestive heart failure (Francis,1990; 1992; Pratt *et al,* 1995).

3.3c. The Sympathetic Nervous System

Abel and Craford (1897) were the first to identify the chemical structure of epinephrine. Langley (1901) noticed the similarity between the effects of injection of extracts of adrenal gland and stimulation of synaptic nerves. A few years later, Barger and Dale (1910) studied the pharmacological aspects of a large series of synthetic amines and termed their action as sympathomimetic. Eliot (1905) suggested that epinephrine might be released from sympathetic nerve endings. It was not until 1921 that the presence of sympathomimetic substances in mammalian sympathetic nerves was shown (Cannon and Uridil, 1921).

During the sympathetic adrenergic response there are two main sources of catecholamines; one is the terminal varicosities of the adrenergic neurons and the other is the adrenal medulla. The neurons release norepinephrine which is synthesized in the varicosities via dopa and dopamine and ultimately

forms tyrosine and is released into the circulation. After its release into the synaptic cleft, the catecholamine is actively transported back to the axon terminal. Norepinephrine, epinephrine and dopamine all contain a catechol ring and an amine group.

Ang-II promotes the release of norepinephrine from terminal neurons and also enhances adrenergic tone by central nervous activation and by facilitation of ganglionic transmission (Antonaccio and Kerwin, 1981). Further Ang-II amplifies the vasoconstriction achieved by α_1-receptor stimulation (Purdy and Weber, 1988). Thus Ang-II has facilitatory adrenergic actions leading to increased activity of vasoconstrictory norepinephrine. This permissive role of Ang-II provides an indirect mechanism for vasodilatory action of ACE-inhibitors. Recently, an increase in plasma norepinephrine has been correlated with left ventricular dysfunction in patients who do not have overt signs of heart failure (Lyons *et al*, 1995).

3.4. Congestive Heart Failure Subsequent to Myocardial Infarction

The necrosis of myocardial tissue secondary to the loss of blood supply leads to an acute myocardial infarction (AMI). Occurence of AMI is facilitated by series of risk factors including activation of the sympathetic nervous system

(such as emotional upset) (Hamill and Khandheria, 1990; Muller *et al,* 1985; Tofler *et al*, 1990; Willich *et al,* 1989; John, 1991). With the progression of AMI, energy metabolism at the cellular in the affected myocardium shifts to anaerobic glycolysis. The cellular level changes following an AMI are reversible if reperfusion occurs in time (Carrol *et al*, 1990; Larnar and Conway, 1989; John, 1991). Continued ischaemic conditions lead to irreversible cell damage and necrosis.

During the early phase of an AMI, neurohormonal systems are activated (McAlpine *et al*, 1988). Increased level of sympathetic activity was initiated by an increase in circulating catecholomine in the early phase of AMI (Gazes *et al*, 1959; Jewitt *et al*, 1969; Karlberg *et al*, 1982). It was also observed that activation subsides in post infarction days (McDonald *et al*, 1969; McMurray *et al*, 1990; Vaughan *et al*, 1990). Activation of other neurohormonal system in an AMI is still not well understood. McAlpine *et al* (1988) proposed that RAS is activated after sympathetic nervous system activation. In another study, Fontana *et al*, 1990, however, found elevated plasma concentrations of Ang-II within a few hours from the time of infarction. The RAS activation may be due to the complications associated with the early phase of MI (Vaney *et al,* 1984). In addition, elevated Plasma renin activity (PRA) in patients with left ventricular dysfunction was reported (Rouleau *et al*, 1993). Plasma concentration of ANP was also found in the early phase of an AMI (Fontana *et*

al, 1990; Ngo *et al*, 1989, 1991, Phillips et al, 1989; Svanegaard *et al*, 1989; Tan *et al*, 1989) and it remains elevated for approximately three weeks in patients with left ventricular dysfunction (Vaughan *et al*, 1990).

3.5. MI and CHF - The Common Pathway

A neurohormonal hypothesis was proposed to explain the progression of CHF (Packer *et al*, 1992a). The neurohormonal system is well correlated with the mortality rate. Ventricular dysfunction after AMI activates neuroendocrine compensatory mechanisms described earlier in this review. At this time, both vasoconstriction and vasodilator systems are activated simultaneously. The effect of the vasoconstrictor system activation such as sympathetic nervous system and RAS activation are detrimental because they cause excessive systemic vascular resistance (Francis et al, 1992; Packer, 1988; Poole-Wilson, 1993; Packer, 1992b; Swedberg *et al*, 1990; DiBianco, 1994). The sympathetic nervous system has been identified by increased plasma level of NE and it has been directly related with progression of the disease and mortality in heart failure. Both α and β adrenergic receptors are also activated. The α-adrenoceptor is responsible for arterial vasoconstriction (ie increase in afterload) and venous vasoconstriction (ie. increase in preload). The β adrenergic receptors are down-regulated by a chronic stimulation of the

sympathetic nervous system producing functional depression in the myocardium. The sympathetic nervous system in the heart has been comprehensively reviewed (Packer, 1990).

The RAS has also been well correlated with disease severity and mortality. The RAS activation produces preload by sodium and water retention and is mediated via aldosterone secretion. It is primarily activated by the interaction with the sympathetic nervous system. Another neurohormonal system that is activated is ANP (Francis *et al*, 1989; Poole-Wilson, 1993; Benedict et al, 1993). Recently, a great deal of interest has been focused on ventricular remodelling as a consequence of AMI (Young, 1993). Ventricular remodelling is a progressive, pathological change in the geometry of the ventricle after AMI. This phenomenon results in redistribution of cardiac loading as the remaining myocardium faces reduction in stroke volume, increase in systolic and diastolic volumes which in turn elevates ventricular wall stress producing thinned and dilated ventricular chamber (Piero *et al*, 1993).

The changes at the cellular level in ventricular remodelling involve infarct expansion which extends to the non-infarcted region of the myocardium. Edema and inflammation occurs in the infarcted region and scar formation takes place within days. Formation of the scar and subsequent volume afterload in the myocardium are the two major pathological alterations

after the coronary occlusion. There are several mechanisms to explain the observed dilation and thinning that occurs in association with infarct expansion (Hutchins *et al*, 1978; Paul, 1995). These include cell rupture, intracellular space reduction, myocyte stretching or slippage of groups of myocytes so that fewer cells get distributed across the wall (Paul, 1995). Patients who undergo infarct expansion often suffer from diminished stroke volume, cardiac output and ejection fraction. Such patients are more likely to experience complications such as CHF (Eaton *et al*, 1979; Erlebacher *et al*, 1982; Schuster and Bulkley, 1979; Jugdutt and Michorowski, 1987; Paul, 1995). Infarct expansion can be studied by echocardiography which shows thinning and elongation of the non-contractile infarcted segment and distortion of the ventricle (Eaton *et al*, 1979; Paul, 1995).

Several investigators have reported that hypertrophy of non-infarcted myocardium occurs in response to the loss of myocardium (Nolan *et al*, 1988; Rubin et al, 1983; Anversa *et al*, 1985a, 1985b; Firth and Dunnmon, 1990). Cardiac hypertrophy may be defined as an increase in the mass of the heart due to increase in volume of the individual cardiac myocytes (Bishop, 1983; Firth and Dunnmon, 1990). Physiologically, hypertrophy of cardiac myocyte also occurs as a result of strenuous exercise. Pathologically, hypertrophy is always associated with chronic severe workload of the heart (Wilkman-Coffelt *et al*, 1979; Firth and Dunnmon, 1990). The hypertrophy of the cardiac myocyte is a

complex event. After MI, diastolic pressure and wall stress increase in proportion to the size of the myocardial infarct. As a result, ventricular performance is markedly depressed (Olivetti *et al*, 1991; Paul, 1995).

In addition, ventricular chambers are enlarged due to anatomical and cellular changes from increased wall stress (Olivetti *et al*, 1991) that leads to volume overload (Grossman, 1980; Paul, 1995). The ventricular chamber enlargement is a compensatory mechanism to accommodate the volume overload and brings the diastolic pressure to normal. A proportional increase is seen in chamber radius but only a slight a increase is observed in wall thickness. Initially, volume overload and increased wall stress are compensated by hypertrophy, but with the progressive left ventricular dilation (LV dilation), LV performance is deteriorated further and clinical heart failure develops (Grossman, 1980; Katz, 1990; Paul , 1995).

4. ACE INHIBITORS

LV dilation following MI may be attenuated by the treatment with an ACE-inhibitor (DiBianco, 1994). In addition, the beneficial effect of ACE-inhibitors can be shown by reduction in preload/afterload with increased ventricular performance (Rich and Moore, 1994). The neurohormonal changes associated with ACE-inhibitor therapy include increased plasma renin,

decreased Ang-II, decreased aldosterone and depressed sympathetic nervous activity (Opie, 1992).

4.1. Mechanism of ACE-inhibitor action

Although it is clear from the experimental evidence that ACE-inhibitor therapy is effective in attenuating ventricular remodelling following MI, its mechanism of action remains unclear.

The immediate effect of administering ACE-inhibitor drugs is a reduction in circulating Ang-II concentration with an associated fall of blood pressure. Inhibition of Ang-II within the circulation causes depression in sympathetic nervous system activity (Julius *et al*, 1991; Mooser *et al*, 1991). It also decreases the rate of aldosterone secretion (Robertson, 1994). Ace-inhibitors benefit a patient by limiting the progression of ventricular remodelling in two ways. First, the ventricular wall stress is reduced by blocking the production of Ang-II. Second, systemic vascular resistance is subsequently decreased, this in turn decreases afterload on the heart and increases cardiac output. ACE-inhibitors also cause venous vasodilation producing a decrease in the cardiac preload which may also help to increase cardiac output (Sami, 1994). ACE-inhibitors can be potentially important to patients who have sustained an AMI (Pfeffer *et al*, 1992; Raya *et al*, 1989;

Sharpe *et al*, 1991; Sami, 1994) by decreasing remodelling and simultaneously decreasing cardiac dilation (Raya *et al*, 1989; Sharpe *et al*, 1991; Sami, 1994).

Some studies have shown that ACE-inhibition limits cardiac enlargement after AMI but these results have not been linked to improved prognosis (Fara *et al*, 1993; Sami, 1994). Several clinical results, however, suggest that ACE-inhibitors have beneficial effects on patients with LV dysfunction following AMI (Pfeffer et al, 1992; ISIS et al, 1993; Cohn *et al*, 1991).

4.2. Limitations of ACE-inhibitor Treatment

Persistent chronic cough is probably the most common clinical problem associated with ACE-inhibitor treatment and is a frequent cause for discontinuation of treatment. Cough is reported to occur in 8 to 25% of patients receiving various ACE-inhibitors. It is reported to be more pronounced in patients on enalapril (25%) than captopril (12%), cilazapril (13%) (Goldszer *et al*, 1988) and perindopril (3-8%) (Lees *et al*, 1989; Swedberg *et al*, 1992).

Another limitation observed with ACE-inhibitors is proteinurea. It was most often seen in patients receiving high doses of captopril. The proteinurea was considered to be due to sulfhydryl group of captopril (Opie, 1992). The

third feared complication of ACE-inhibitor therapy in CHF patients is hypotension (Opie, 1992). Captopril produced hypotension at 1.5 hour after the first dose of 2.5 mg/day (Opie, 1992). Surprisingly, perindopril did not seem to cause hypotension after the first dose of 2 mg/day. (Sami, 1994; MacFayden *et al*, 1991).

Finally, renal function deterioration is sometimes observed with the use of ACE-inhibitors. Thus, in CHF where renal function is already limited with poor renal blood flow, it may be difficult to introduce the ACE-inhibitor therapy. These drawbacks of ACE-inhibitors have lead investigators to search for new and improved drugs.

4.3. Quality of Life

The quality of life is an important consideration in the treatment of CHF. Patients on ACE-inhibitor therapy appear to have a better quality of life than those receiving other antihypertensive treatments (Croog *et al.* 1986). Patients under ACE-inhibitor therapy performed better, had fewer nervous system problems and had a higher well being score. Some patients, however, suffered from the adverse side effects described earlier.

More recently, a number of new ACE-inhibitors became available including lisinopril, perindopril, ramipril, quinapril and cilazapril and they all

have a common property of reducing the blood pressure when given as a monotherapy in CHF. Imidapril is a recently reported ACE-inhibitor drug which promises to be very effective with fewer side effects than other ACE-inhibitor drugs.

5. THE ACE-INHIBITOR - IMIDAPRIL

The rationale for the development of new ACE-inhibitors is based on the premise that the new drug will achieve the same goals as their predecessors as well as offer additional advantages. The structure of Imidapril hydrochloride is(TA-6366I)(45)-1-methyl-3-{(28)-2-[N-((15)-1-ethoxycarbonyl-3-henylypropyl) amino] propionyl }-2-oxo-imidazolidine-4-carboxylic acid (Yamada *et al*, 1992a). Figure 3. shows the structure of active and inactive metabolite of ACE-Inhibitor Imidapril. Imidapril is an ester prodrug that is rapidly converted to active diacid (6366A) *in vivo* by metabolism. The ACE-inhibiting effect of Imidapril in the heart, when compared to enalapril and another ACE-inhibiting drug, was longer and stronger after the same dose of oral administration in rats (Ogiku *et al*, 1994). Imidapril also reduces mortality more significantly than enalapril (Vandenburg *et al*, 1994). Thus, Vandenburg

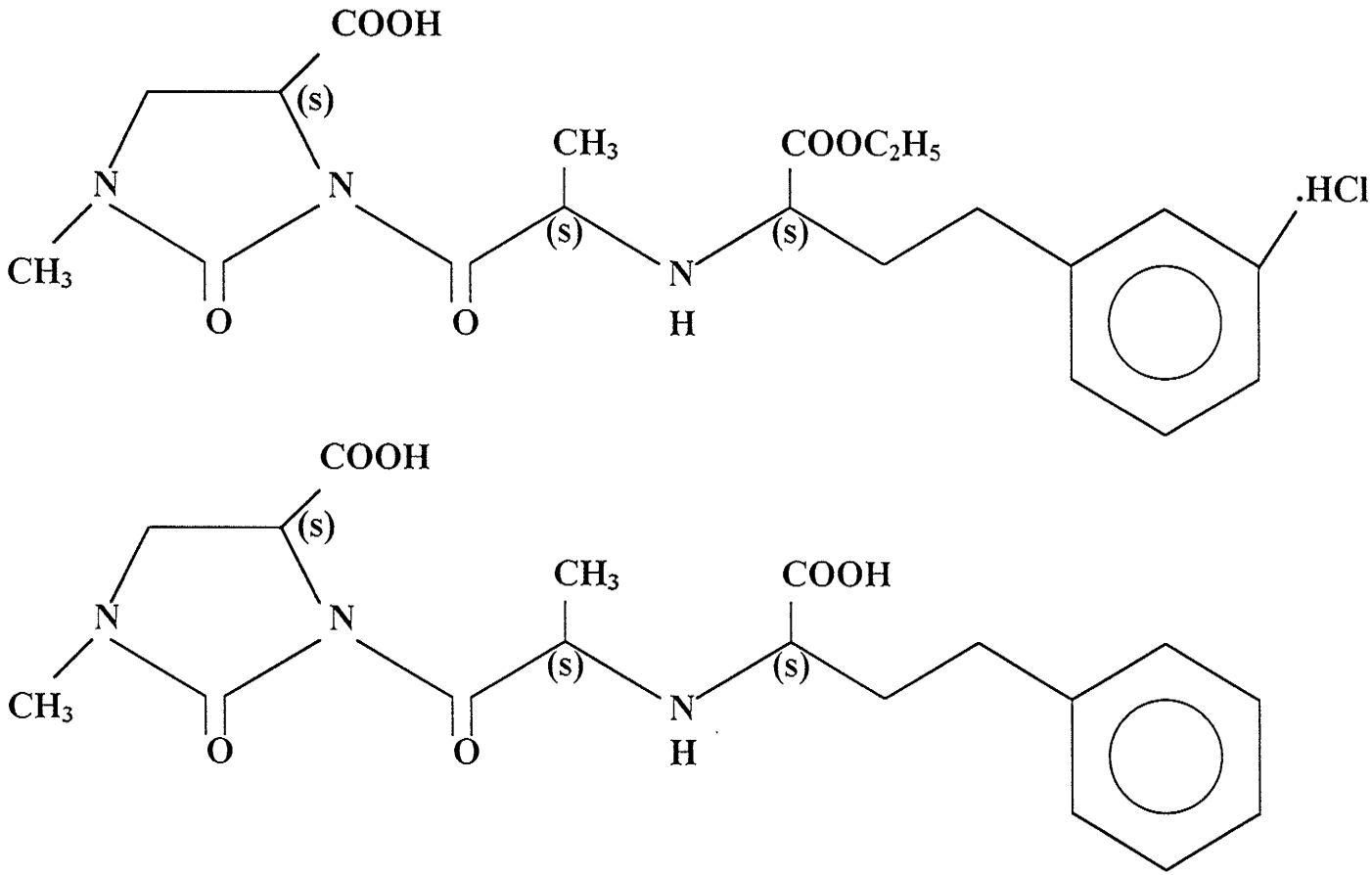


Figure 3. Structures of Imidapril (TA-6366, I) *(top)* and its active metabolite (6366A, II) *(bottom)*. (Compiled from Kubo et al., 1992)

et al concluded that Imidapril was slightly more potent than enalapril and much more potent than captopril in duration of the ACE-inhibiting action.

5.2. Absorption, distribution and elimination

Imidapril is readily absorbed upon oral administration and the site of absorption is the entire gastrointestinal tract. The drug is converted by liver esterases to Imidaprilat, which is the active form of the drug. The orally administered drug is absorbed 62% in rats and 38% in dogs (Yamada *et al*, 1992a). Generally, food had little effect on the absorption of the drug by the gastrointestinal tract (Yamada *et al*, 1992b).

Once the drug is absorbed, the ester prodrug is rapidly metabolized to its active form, Imidaprilat. Thus, Imidapril is found in the serum for a very short period. In contrast, the distribution of enalaprilat is more prolonged. Yamada *et al* (1992a) showed that the disappearance of the Imidapril drug was much faster in rats than dogs. He demonstrated that the plasma levels of the active metabolite, Imidaprilat, reached a peak at 1-2 hours in rats and 2-3 hours in dogs after oral administration and declined with a half life of 0.9 hours in rats and seven hours in dogs.

Most of the Imidapril administered orally or intravenously was eliminated. The orally administered Imidapril in rats and dogs showed

elimination of about 36-38% of the dose in the urine and 57-62% in the feces within 96 hours. While intravenous administration showed 69-73% elimination in the urine and 24-32% in the feces of both rats and dogs within 96 hours (Yamada *et al*, 1992a).

Later, tissue distribution and metabolic profiles of Imidapril [N-methyl [^{14}C] 1 and 5 mg/kg or Imidapril [alanine 3-^{14}C] 1 mg/kg were studied in both male and female rats after oral and intravenous administration. Imidapril was found to be distributed in all tissues except the CNS. Maximum concentration was observed in most tissues at 30 minutes to one hour after dosing. The Imidapril concentrations in kidney, liver and lung were higher than its concentration in plasma (Yamada *et al*, 1992c). Authors also reported the slow disappearance of radioactivity from the lung is a consequence of its high affinity for ACE (present in lung). In addition, studies were performed to assess the dose of Imidapril (Vandenburg *et al*, 1994). The dose administered to patients was 5, 10 and 20 mg with progressively better results. It was concluded that 10 mg is the best dose to give the patient, but some individuals can tolerate and benefit from 5 mg or 20 mg.

5.3. Limitation of the ACE-inhibitor, Imidapril

The most common and irritating side effect of previous ACE-inhibitor treatment - the cough - was not observed with Imidapril. However, with Imidapril treatment, headache was frequently experienced by the patients.

Another frequently occurring adverse effect with Imidapril therapy was dizziness. In addition, in several other studies done on patients treated with Imidapril the incidence and types of adverse effects associated with Imidapril have not changed significantly (Yamada *et al*, 1992a,b).

III. MATERIALS AND METHODS

1. MATERIALS

[γ ^{32} P] ATP (specific activity of 10 Ci/mmole) and [^{3}H]- PtdIns (4,5) P_2 [inositol-2-[^{3}H] (N)- (5.45 Ci/mmole) were purchased from DuPont Canada Inc./New England Nuclear (Mississauga, Ont., Canada]. Alamethicin, sodium cholate and unlabelled adenosine triphosphate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Nonlabelled PtdIns $(4,5)_2$ was purchased from Calbiochem Co. (La Jolla, CA, USA). Dowex IX (formate form, 100-200 mesh) was obtained from BioRad Laboratories (Mississauga, Ont, Canada). All other reagents were of analytical grade or of highest grade available.

2. METHODS

2.1. SURGICAL PROCEDURE FOR THE INDUCTION OF CHF IN EXPERIMENTAL MODELS

CHF following myocardial infarction was produced in male Sprague-Dawley rats of 150-175 g weight by occlusion of the left anterior descending coronary artery (Dixon *et al*, 1990). The animals were anesthetized with isofluorine and an incision was made along the left sternal border. The fourth rib was cut proximal to the sternum and the retractors were inserted. The pericardial sac was perforated and the heart was exteriorized through the intercostal space. The left coronary artery was ligated with a silk suture (6-0) about 2 mm from its origin. The heart was repositioned in the chest and the incision was closed with 3-0 suture. Throughout the course of surgery, rats were maintained on a positive pressure ventilator delivering a mixture of 95% O_2 and 5% CO_2 mixed with isofluorine. Closure of the wound was accomplished by purse string suture. The mortality rate of all animals operated in this manner was about 35% within 48 hours. The sham operated animals served as controls and the same procedure was performed with the exception

that the suture around the coronary artery was not tied. The animals were allowed to recover and maintained on food and water *ad libitum*. A new set of sterilized surgical instruments was used for each animal. All the surgical instruments were sterilized. Atria and scar tissue was removed and both ventricles ventricles were used for isolation of purified sarcolemmal membranes. Right and viable left ventricle were used separately for the isolation procedures. Ventricles were weighed to determine ventricle to body weight ratio.

2.2. Drug dosage

Imidapril (1mg/kg of the body weight) was administered to animals daily beginning from the week 5 after the surgery till the end of week 8. The drug was dissolved at a concentration of 1mg/mL in the tap water. Animals were weighed and the required dosage was calculated according to the body weight. The dissolved drug was administered orally with the help of a gavage tube.

2.3. Isolation of Sarcolemmal membrane and the Cytosol fraction

The isolation procedure were carried out at 4° C. The rats were decapitated, in accordance to animal care committee, University of Manitoba. After dissection, the heart was rapidly removed from the body and the atria and connective tissues were removed and the left and right ventricles were separated. Sarcolemmal membranes from the ventricles were isolated according to the Pitts procedure (Pitts, 1979). The right ventricles were frozen in liquid nitrogen and stored at −70° C. The left and the right ventricles were separated since Phosphoinositide specific PLC activity in the left ventricle is upregulated while in the right ventricle it is downregulated.

The ventricles were washed with Pitts solution 1 (0.6 M sucrose, 10 mM Imidazol, pH 7.0) to remove excess blood and then the ventricles were minced with scissors in solution 1 (0.6 M sucrose, 10 mM Imidazol, pH 7.0). The ventricles were homogenized with the Polytron PT-20 (6×15s) and the homogenate obtained was centrifuged at 12,000g for 30 min. After centrifugation, the supernatant was collected in a cylinder whereas pellet were discarded. A portion (.5 ml) of the supernatant was centrifuged at 100,000g for 60 min to obtain the cytosolic fraction, which was divided into equal aliquots, frozen in liquid nitrogen and stored at −70° C for later use.

The remaining supernatant was diluted with solution 2 (20 mM Mops/KOH, 160 mM KCl, pH 7.4) and centrifuged at 100,000g for 60 min. From the second centrifugation step, supernatant was discarded and the pellet was resuspended in solution 2 (20 mM Mops/KOH, 160 mM KCl, pH 7.4) and layered over solution 3 (100 mM Tris/HCl, 50 mM Na_2 pyrophosphate, 300 mM KCl, 30% sucrose, pH 8.3) and centrifuged (using Beckman swinging bucket rotor) at 100,000g for 90 min. The resultant band at sucrose buffer interface, was taken and diluted with 3 volume of solution 2 (20 mM Mops/KOH, 160 mM KCl, PH 7.4) and then centrifuged at 100,00 for 30 min. The resultant pellet rich in sarcolemmal membrane was resuspended in solution 4 (250 mM sucrose, 10 mM histidine PH 7.4) and was divided into equal aliquots, frozen in liquid nitrogen and stored at –70° C.

2.4. Phospholipase C assay

Phospholipase C activity, associated with the sarcolemmal and the cytosolic fractions, was assayed according to the method of Meij *et al.*(1992). The substrate were prepared by mixing an aliquot of ^{3}[H] PtdIns(4,5)P_2 with an aliquot from 1mM stock solution (in chloroform) of the cold substrate. The mixture was evaporated to dryness under a stream of nitrogen and redissolved

in 10% Na cholate (232 mM). The substrate solution was kept under nitrogen gas overnight at 0-4°C and diluted to 160 μM substrate/ 112 mM Na cholate shortly before addition to the incubation mixture.

The reactions were carried out at 37°C in a mixture containing 30 mM HEPES-Tris (PH 7.0), 100 mM NaCl, 2mM EGTA, 3.13 mM $CaCl_2$ ([free Ca^{2+}] = 1mM), 15 μg sarcolemmal or cytosolic proteins, 14 mM Na-cholate and 20 μM [^{3}H]PtdIns (4,5)P_2 in a final volume of 40 μL. The reaction was terminated after 2.5 min by the addition of 144 μL stop solution: ice-cold chloroform: methanol: HCl (1:2:0.2) to each sample. The control reaction was carried out under identical conditions except the protein was added after the addition of the stop solution. The phase were separated by adding 48 μL of 2M KCL, and 48 μL chloroform vortexed for 30 sec followed by 5 min centrifugation in a microcentrifuge. The upper phase from each sample was aspirated and applied to a 300 μL column of Dowex 1X8 (formate from 100-200 mesh) together with 0.75 ml water. The inositol mono-, bis-, and trisphosphate were eluted by consecutive washes of the column with 1 ml 0.1 M formic acid containing 0.2 M, 0.4 M and 1M NH_4 formate respectively. The labelled products were determined by liquid scintillation counting. A known quantity of the substrate was counted for determination of the specific activity of the substrate.

2.5. Protein Determination

Protein concentrations were determined in the sarcolemmal membrane samples and cytosolic fractions according to Lowry et al. (1951), using bovine serum albumin (fraction V) as a standard.

2.6. Statistical analysis

All experiments were performed in quadruplicate. Results are presented as means ± SEM. Statistical analysis were done using one sided, paired Student t-test. A value of $P<0.05$ was considered significant.

IV. RESULTS

1. Viable left ventricle/body weight ratio in moderate stage of CHF.

A increase of 28% in the viable left ventricular (LV) mass/body weight ratio in 8 week MI animals was observed compared to control values, and this change was significant ($P<0.05$). The ratio has been used to indicate the degree of mammalian LV hypertrophy (Dixon *et al.*, 1990), the greater the ratio the greater the extent of hypertrophy. Baker *et al.* (1992) has shown that the RAS plays an important role in modulating the adaptive growth pattern in cardiac hypertrophy. Regression of cardiac hypertrophy can be produced by clinical intervention that can cause regression of cardiac hypertrophy through the inhibition of RAS (Makino *et al.* 1993). In the present study, animals were treated with the ACE-inhibitor imidapril from the beginning of fifth week to the end of eighth week. The imidapril treated MI animals exhibited a significant (14%, $P<0.05$) increase in LV/body weight ratio as compared to treated sham control. While imidapril treated experimentally induced MI animals showed a significant decrease of 12% ($P<0.05$) in LV/body weight ratio in comparison to the untreated myocardial infarcted group (Table1).

Table 1. Viable left ventricular / body weight ratio in 8 weeks post infarct animals.

	Viable LV weight./ Body weight (mg/g)
Sham	1.84 ± 0.14
MI	2.37 ± 0.24 *
Imidapril treated sham	1.79 ± 0.04
Imidapril treated MI	2.08 ± 0.03 #

The values are expressed as the means ± SEM of five different experiments. In case of MI group, it was remaining viable left ventricular (LV) weight upon removal of the scar tissue and in sham it was the whole left ventricle weight.

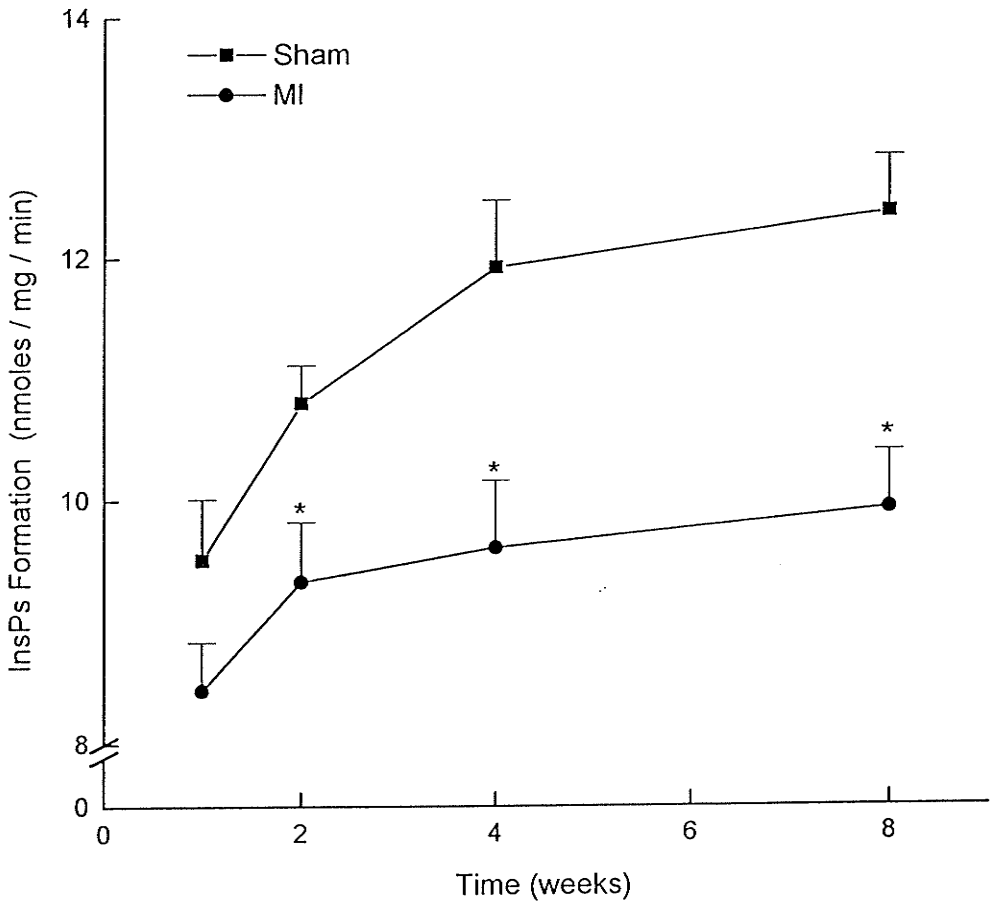
* Significantly ($P<0.05$) different from sham control values.
# Significantly ($P<0.05$) different from values derived from untreated MI group.

2. Sarcolemmal phosphoinositide phospholipase C activity in CHF.

PLC is activated by receptors via G protein to hydrolyse PtdIns(4,5)P_2 , which in turn generates two second messengers, DAG and IP_3. The second messengers perform a number of functions as previously mentioned in the section entitled "Review of Literature".

Figure 4 shows the time dependent PtdIns(4,5)P_2 hydrolysis by the sarcolemmal phosphoinositide PLC in experimentally induced MI and sham control animals at 1, 2, 4, and 8 weeks post surgery. The PI-PLC activity examined at each time point was decreased in the MI group. This decrease in the PLC activity at 2,4, and 8 weeks was 17, 19, and 23% ($P<0.05$) respectively. Since maximal changes in the PI-PLC activity was seen at 8 weeks, the formation of different inositol phosphate InsP, $InsP_2$, and $InsP_3$ was analyzed at this time point. InsP is significantly ($P<0.05$) depressed in MI as compared to sham control (Figure 5), similar conclusion was reached by analyzing $InsP_2$ (Figure 6) and $InsP_3$ (Figure 7).

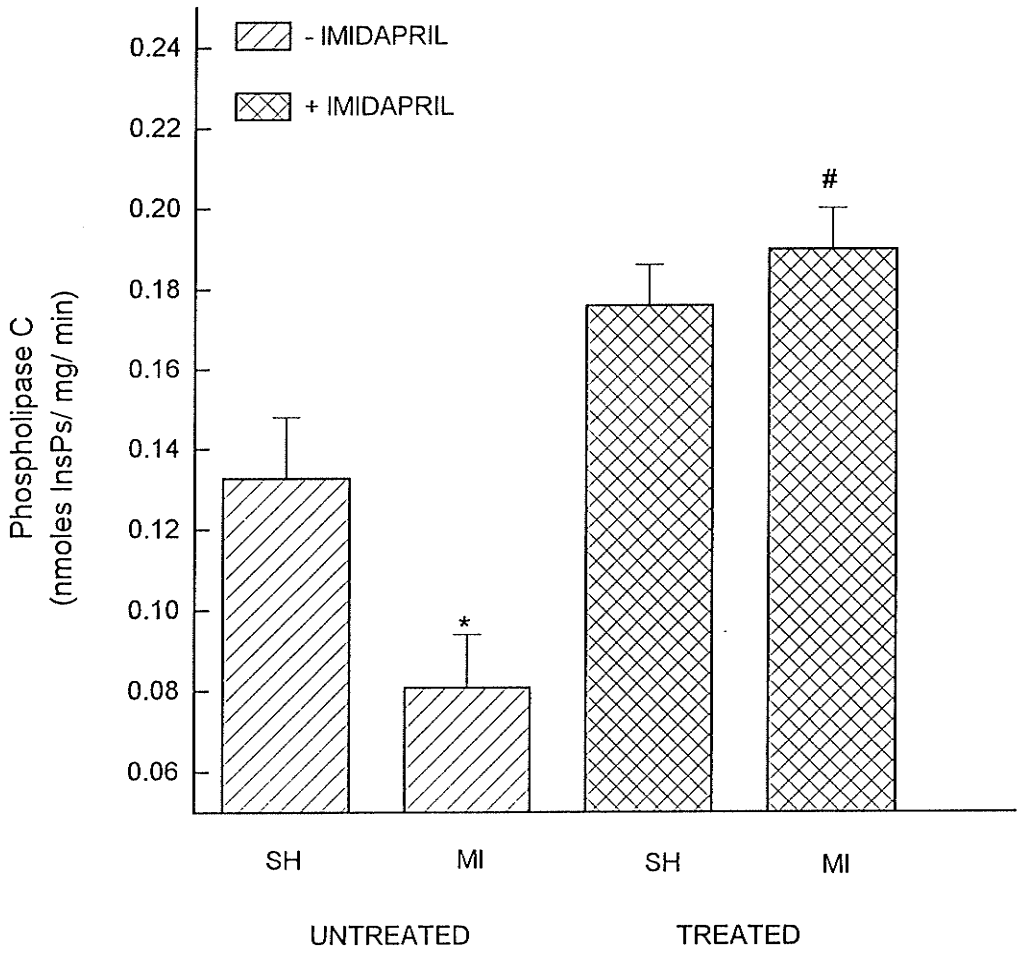
Figure 4. Time dependent phosphatidylinositol bisphosphate hydrolysis by sarcolemmal phosphoinositide phospholipase C activity in myocardial infarcted and sham control rats.



The values are means ± SEM of three to five experiments. Assays were performed in quadruplicate. Abbreviations: InsPs = total inositol phosphates, MI=myocardial infarction.

* Significantly ($P<0.05$) different from the corresponding sham control values.

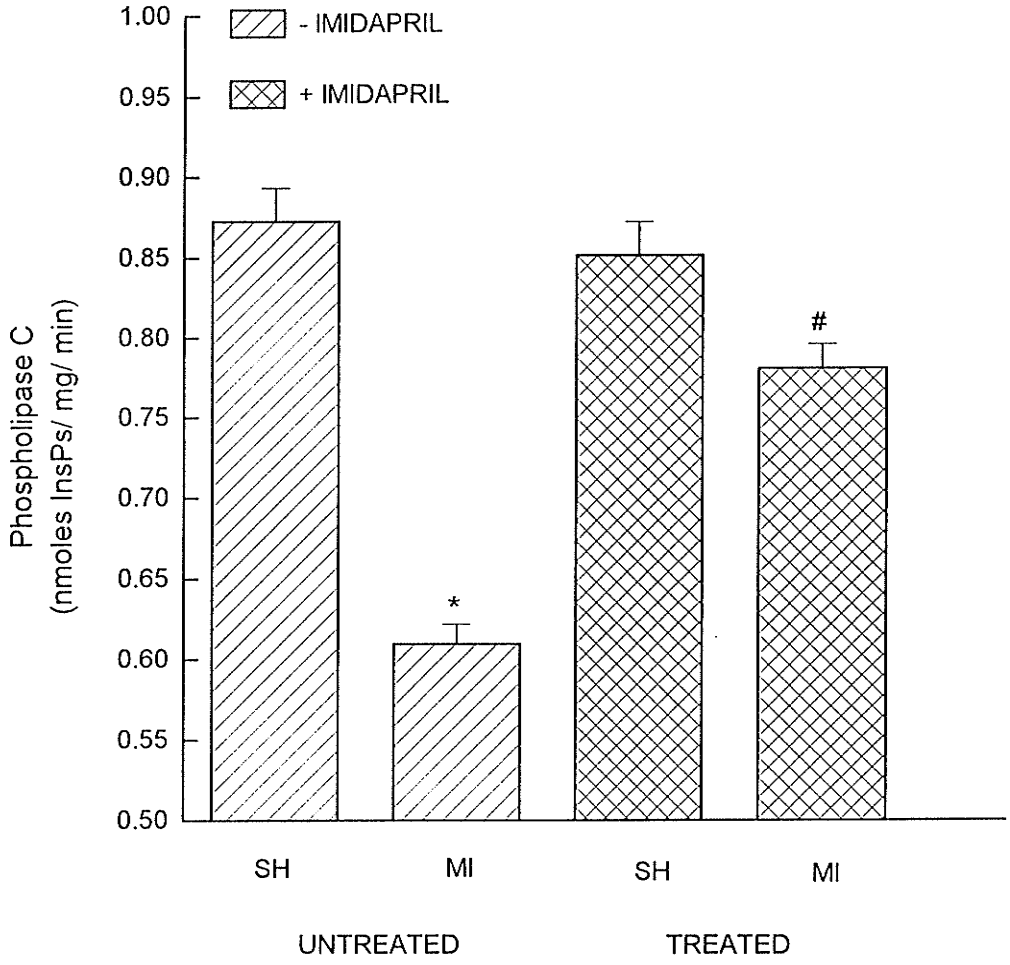
Figure 5. Formation of inositol monophosphate by sarcolemmal phosphoinositide phospholipase C in week 8untreated and imidapril treated myocardial infarcted and sham control rats.



Sarcolemmal phospholipase C activity of week 8 myocardial infarcted (MI) and sham control (SH) rats in the absence and presence of imidapril (1 mg/kg/day) was assayed under standard conditions as described in "MATERIALS AND METHOD" in the presence of 20 μM [^{3}H] PtdIns (4,5)P_2 for 2.5 min at 37° C. Results are means ± SEM of five experiments. Assays were performed in quadruplicate.

* Significantly ($P<0.05$) different from corresponding sham control values.
# Significantly ($P<0.05$) different from values for untreated MI.

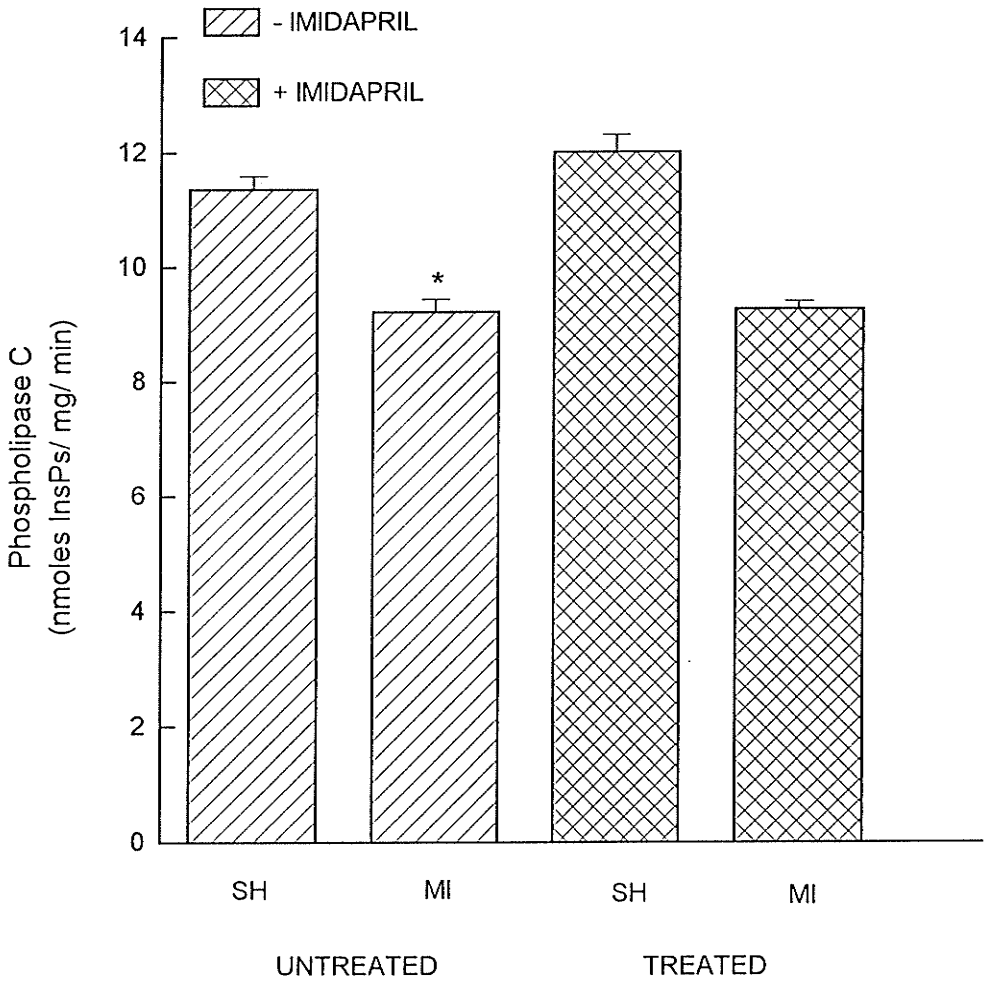
Figure 6. Formation of inositol bis (1, 4) phosphate by sarcolemmal phosphoinositide phospholipase C in week 8 untreated and imidapril treated myocardial infarcted and sham control rats.



Sarcolemmal phospholipase C activity of week 8 myocardial infarcted (MI) and sham control (SH) rats in the absence and presence of imidapril (1 mg/kg/day) was assayed under standard conditions as described in "MATERIALS AND METHOD" in the presence of 20 μM [^{3}H] PtdIns (4,5)P_2 for 2.5 min at 37° C. Results are means ± SEM of five experiments. Assays were performed in quadruplicate.

* Significantly ($P<0.05$) different from corresponding sham control values.
# Significantly ($P<0.05$) different from values for untreated MI.

Figure 7. Formation of inositol tris (1, 4, 5) phosphate by sarcolemmal phosphoinositide phospholipase C in week 8 untreated and imidapril treated myocardial infarcted and sham control rats.



Sarcolemmal phospholipase C activity of week 8 myocardial infarcted (MI) and sham control (SH) rats in the absence and presence of imidapril (1 mg/kg/day) was assayed under standard conditions as described in "MATERIALS AND METHOD" in the presence OF 20 μM [^{3}H] PtdIns (4,5)P_2 for 2.5 min at 37° C. Results are means ± SEM of five experiments. Assays were performed in quadruplicate.

* Significantly ($P<0.05$) different from corresponding sham.

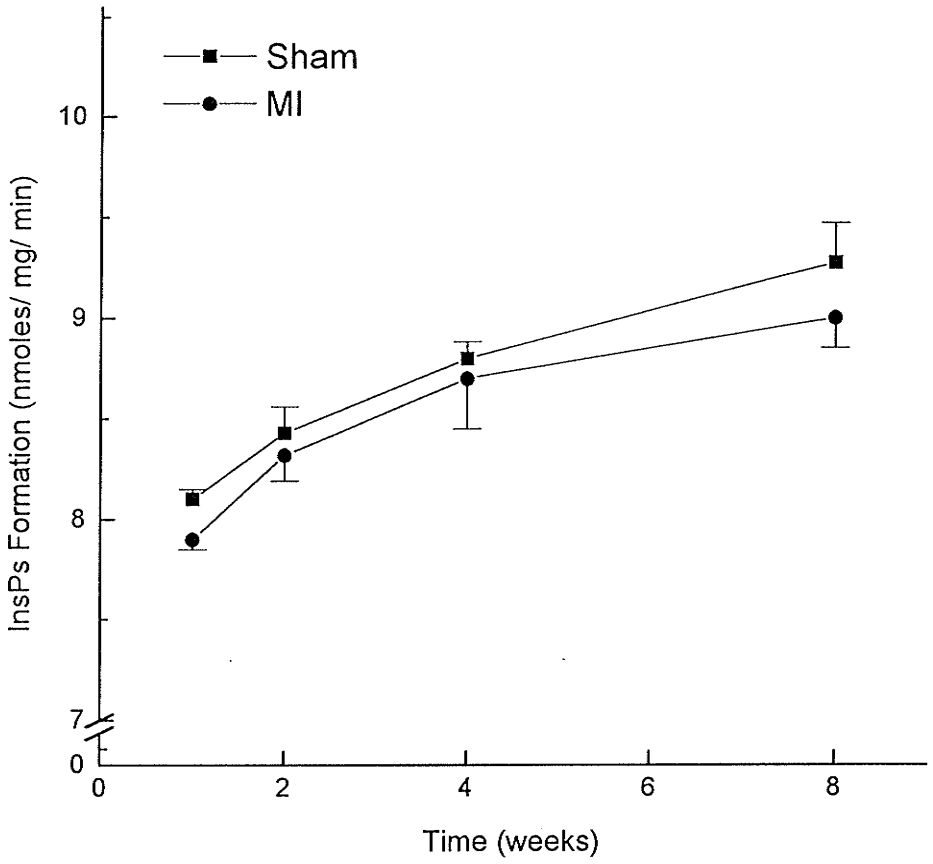
3. Cytosolic phosphoinositide phospholipase C activity in CHF

The cytosol was obtained from the same experimental group from which the sarcolemma was isolated. Figure 8 illustrates the time dependent PtdIns(4,5)P_2 hydrolysis by the cytosolic PI-PLC in experimentally induced MI and sham control 8 week animals. The PI-PLC activity observed at 1, 2, 4, and 8 weeks showed no significant difference in MI, as compared to sham control animals. Table 2 shows the formation of different inositol phosphate species by cytosolic PI-PLC in 8 week animals. The data obtained demonstrates no significant change in inositol phosphate species suggesting that no rearrangement in InsP, $InsP_2$, and $InsP_3$ distribution occurs in MI.

4. Sarcolemmal phosphoinositide phospholipase C activity in Imidapril treated 8 week MI animals

ACE-Inhibitor treatment is recommended after four weeks of appearance of symptoms of CHF, when patients suffer from low ejection fraction and symtomatic cardiac failure. Also, the treatment is less effective, if the treatment is given right after infarction and especially if there is

Figure 8. Time dependent phosphatidylinositol bis phosphate hydrolysis by cytosolic phosphoinositide specific phospholipase C in myocardial infarcted and sham control rats.



The values are means ± SEM of three to five experiments. Assays were performed in quadruplicate. Abbreviations: InsPs=total inositol phosphates, MI= myocardial infarction.

Table 2. Formation of different inositol phosphate species by cytosolic phosphoinositide phospholipase C in week 8 myocardial infarcted and sham control rats.

	Inositol Phosphate Species		
	InsP	$InsP_2$	$InsP_3$
	(nmole. min^{-1} .mg $protein^{-1}$)		
Sham	0.140 ± 0.024	0.499 ± 0.038	8.64 ± 0.135
MI	0.148 ± 0.024	0.473 ± 0.032	8.38 ± 0.141

Cytosolic Phospholipase C activity was assayed under standard conditions as described in "MATERIALS AND METHODS" in the presence of 20 μM [^{3}H]- PtdIns(4,5))P_2 for 2.5 min at 37° C . Values are means ± SEM of inositol phosphate formation in five experiments. Assays were performed in quadruplicate. Abbreviations: InsP= inositol 1-phosphate, $InsP_2$= inositol 1,4-bisphosphate, $InsP_3$=inositol 1,4,5-trisphosphate, InsPs= total inositol phosphates, MI= myocardial infarction.

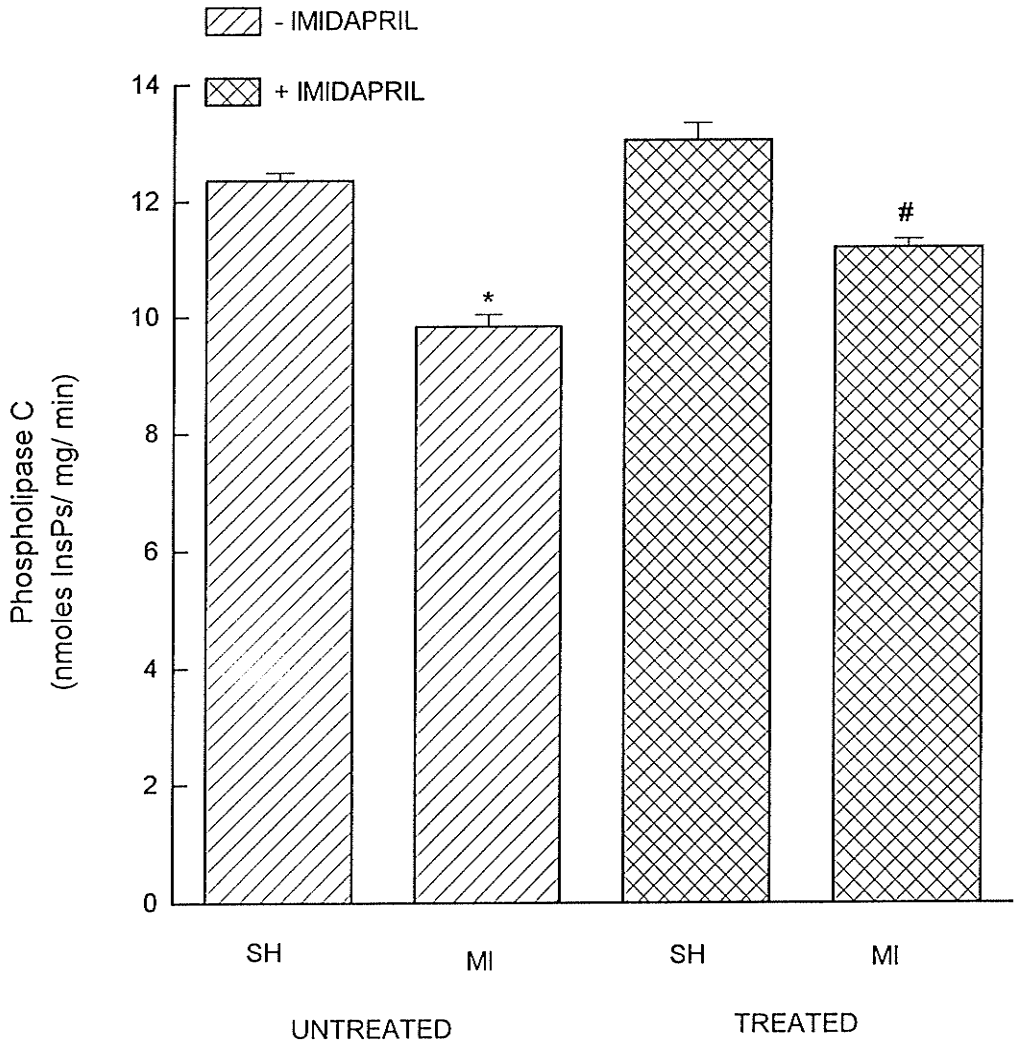
evidence of ongoing ischemia (Ball, 1993). The animals were treated with imidapril, at a dose of 1 mg/ kg/day from the beginning of the fifth to the end of the eighth week.

Sarcolemmal PI-PLC activity in untreated 8 week MI animals was significantly depressed (23%, $P<0.05$) in comparison to sham control animals (Figure 9). However, after treatment with Imidapril a significant, but partial recovery (9%, $P<0.05$) was observed. The PI-PLC activity in the treated MI group was depressed by 16% in comparison to untreated sham control, representing an improvement of 9%. The greatest difference in PI-PLC activity was observed in the 8 week animals as this is mild to moderate stage of CHF. The formation of different inositol phosphate species in 8 week MI and sham control animals were analyzed. The InsP showed a significant recovery ($P<0.05$) in imidapril treated animals as compared to untreated MI (Figure 5), similarly $InsP_2$ also a significant recovery ($P<0.05$) (Figure 6). While $InsP_3$ level did not show a significant recovery in Imidapril treated MI as compared to experimentally induced MI animals (Figure 7).

5. Cytosolic phosphoinositide phospholipase C activity in Imidapril treated 8 weeks MI animals.

The PI-PLC activity was measured in MI animals after Imidapril treatment which commenced from the beginning of fifth to the end of eighth

Figure 9. Sarcolemmal phospholipase C activity in untreated and Imidapril treated 8 weeks myocardial infarcted and sham control rats.

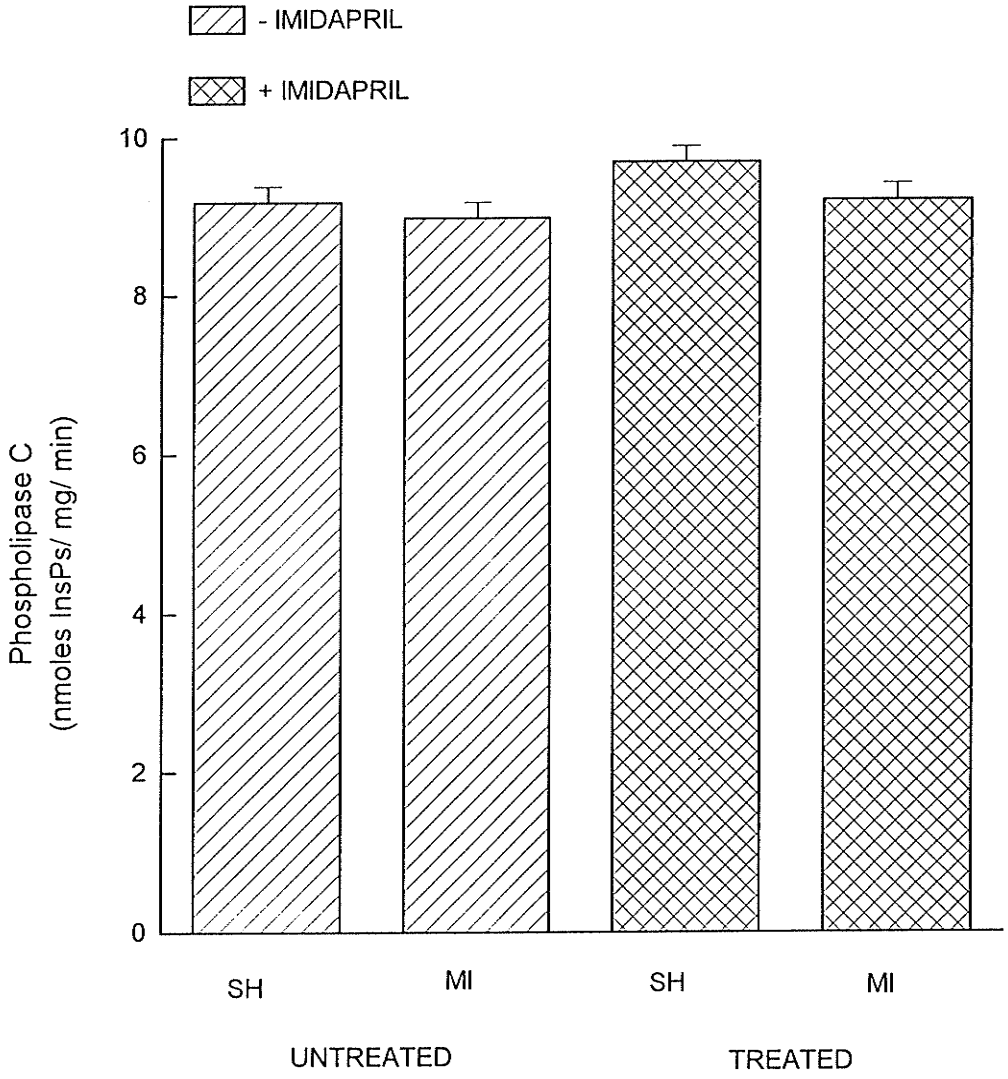


Sarcolemmal Phospholipase C activity of week 8 sham control (SH) and myocardial infarction (MI) rats in the absence and presence of Imidapril (1 mg/kg 0f the body weight) was assayed under standard conditions as described in "MATERIALs AND METHOD" in the presence of 20 μM [^{3} H] PtdIns (4,5)P_2 for 2.5 min at 37° C. Results are mean ± SEM of five experiments. Assays were performed in quadruplicate.

Significantly ($P<0.05$) different from untreated MI.
* Significantly ($P<0.05$) different from corresponding sham.

week after occlusion of coronary artery. Cytosolic PI-PLC activity in untreated and imidapril treated MI and sham control animals is shown in Figure 10. imidapril treatment caused about 5% increase in cytosolic PI-PLC activity as compared to untreated MI was observed, however, it was found not to be significant. A breakdown of the inositol phosphate species formed by the cytosolic PI-PLC are shown in Table 3. It can be seen that no significant differences in inositol phosphate were observed suggesting that no rearrangement of these species occurs in MI.

Figure 10. Cytosolic phospholipase C activity in untreated and Imidapril treated 8 week myocardial infarcted and sham control rats.



PLC activity in week 8 sham control (SH) and myocardial infarction (MI) rats in the absence and presence of Imidapril (1 mg/kg of body weight) was assayed under standard conditions as described in "MATERIALS AND METHODS" in the presence of 20 μM [^{3} H]-PtdIns (4,5)P_2 for 2.5 min at 37° C. Results are the means ± SEM of four to five experiments with quadruplicate determinations.

Table 3. Formation of different inositol phosphate species by cytosolic phospho-inositide phospholipase C in week 8 Imidapril treated myocardial infarcted and sham control rats.

	Inositol Phosphate Species		
	InsP	$InsP_2$	$InsP_3$
	(nmole. min^{-1} .mg $protein^{-1}$)		
Sham Imidapril treated	0.215 ± 0.032	0.415 ± 0.031	9.15 ± 0.130
MI Imidapril treated	0.184 ± 0.036	0.439 ± 0.039	8.60 ±.227

Cytosolic Phospholipase C activity was assayed under standard conditions as described in "MATERIALS AND METHODS" in the presence of 20 μM [^{3}H]- PtdIns(4,5))P_2 for 2.5 min at 37° C . Values are means ± SEM of inositol phosphate formation in five experiments. Assays were performed in quadruplicate. Abbreviations: InsP= inositol 1-phosphate, $InsP_2$= inositol 1,4-bisphosphate, $InsP_3$=inositol 1,4,5-trisphosphate, InsPs= total inositol phosphates, MI= myocardial infarction.

V. DISCUSSION

The present study was undertaken to test the hypothesis that phosphoinositide phospholipase C activity is altered (depressed) and that imidapril treatment restores the phospholipase C activity. The sarcolemmal phosphoinositide phospholipase C activity was depressed significantly in MI as compared to sham controls. However, cytosolic phosphoinositide phospholipase C showed no significant changes. Upon imidapril treatment sarcolemmal phosphoinositide phospholipase C activity was corrected partially while cytosolic phosphoinositide phospholipase C activity had no effect.

1. Rat Model of CHF

The CHF model subsequent to MI was used to see the effect of the ACE-inhibitor imidapril, on Phosphoinositide specific phospholipase C activity. The CHF rat model used in the present study has been studied in research extensively. Although the rat model is not the same as in humans as it lacks collateral circulation, this model is still preferred over other experimental systems by virtue of its reproducibility of the infarct size and comparatively modest cost. Inducing large infarction with ensuing time - dependent

development of CHF in a large number of surviving animals is a lengthy and the cost prohibitive process. Several investigators have studied structural and functional characteristics of viable myocardium subsequent to MI (Anversa *et al.*, 1985a b; Afzal and Dhalla, 1992; Dixon and Dhalla, 1990; Hill and Singal, 1996). The left coronary artery ligation produced 15-45% infarction in the left ventricle of 8 week animals. CHF in mild to moderate stages is associated with hypertrophy of the remaining LV of the heart.

2. Viable LV/ body weight ratio in moderate stage of CHF

The increase in the viable LV/body weight ratio observed in the 8 week animals, is indicative of cardiac hypertrophy in the left ventricle of these animals. The hypertrophy of the heart is a phenomenon in response to increased afterload due to systemic hypertension as well as other clinical conditions causing an increase in the workload of the heart. Cardiac hypertrophy is accompanied by an increase in its contractile force maintaining cardiac function. This is an adaptive mechanism associated with myocardial infarction but can also result in sudden death (Yazaki *et al.*, 1995). Neurohormones such as Ang-II, ANP, and sympathetic nervous system are activated with in a few hours of infarction of the left ventricular wall.

This results in thinning and dilation of the infarcted tissue leading to hypertrophy of the surviving myocardium. The progressive thinning and dilation ultimately results in cardiac dysfunction (Cody, 1994). In the present study there was a significant increase (22%, $P<0.05$) in the ratio indicating hypertrophy of the myocardium. Since no correction was made for the lost myocardium in the infarcted heart, the 22% increase in the ratio seen in the MI group is in fact, an underestimation of the extent of hypertrophy seen in this group (Dixon *et al.,* 1990). In any case, hypertrophy of the viable myocardium in the MI group was established in this study.

Further more, the adaptive mechanism of hypertrophy may be modulated by ACE-inhibitor treatment. A significant reduction in the LV/body weight ratio in the imidapril treated MI group suggested a mitigation of the remodelling process which is suggested to take place in the untreated MI animals.

3. Sarcolemmal and cytosolic phosphoinositide specific phospholipase C activity in Imidapril treated MI animals

ACE-inhibitors are beneficial in both mild to moderate stages of heart failure. In this pathological state, Ang-II stimulates the breakdown of plasma membrane inositol phospholipids by activation of their specific receptors (Kawaguchi and Kitabatake, 1995). Upon receptor stimulation, PtdIns $(4,5)P_2$

yields $InsP_3$ and DAG. $InsP_3$ mobilizes intracellular Ca^{2+} stores while DAG activates PKC (see literature review). Imidapril is a cardioprotective agent in terms of improving the calcium homeostasis and cardiac contractility (Ball, 1993; Brunner *et al.*, 1994). Thus, the present study was undertaken to examine whether the improved cardiac calcium handling and cardiac contractility observed in previous studies, is a consequence of an improvement in the depressed PLC activity observed in CHF.

Phosphoinositide specific PLC activity was examined in coronary ligated animals at 1, 2, 4, and 8 weeks after surgery. A decrease in sarcolemmal PLC activity was observed at each of the time points examined with little or no effect observed in the cytosolic PI-PLC activity. The depression in polyphosphoinositide synthesis in the left ventricle of CHF induced animals has been also been documented in previous reports from our laboratory (Meij *et al.*, 1991; Panagia *et al.*, 1995). Dixon and Dhalla (1991), have reported an increase in contractile function of isolated and perfused heart from CHF induced rat in response to α_1-adrenoceptor stimulation.. These authors reported a significant depression of PLC activity in CHF and suggest that the hydrolysis of PtdIns $(4,5)P_2$ by PLC enzyme in sarcolemma becomes defective. Thus the increased α_1-adrenoceptor response may be occurring through some other pathway. In support of the findings of present study of unaltered cytosolic

PLC activity, a similar conclusion was reached by Shoki *et al.*, (1992). These authors compared stroke prone spontaneously hypertensive rat (SHRSP) with age matched Wistar kyoto rat (WKY) which showed that at week 5 and 10 of hypertrophied heart no significant differences in cytosolic PLC occurred.

The mechanism(s) involved in altering PLC activity are unknown. However, from the known biochemical and physiological modifications which occur in CHF, certain suggestions could be put forward. The neurohormonal system, RAS, sympathetic nervous system and ANP, are activated by initial heart injury, thus exacerbating the haemodynamic abnormalities. The alterations in the neurohormones, to some extent, are proportional to the severity of heart failure.

The increased level of catecholamine causes oxidative damage. The oxidation of catecholamine results in the formation of toxic substances such as aminochromes (eg. adrenochrome and partially reduced forms of oxygen, like hydrogen peroxide, which impairs cardiac contractile function) (Singal *et al.*,1982; Dhalla *et al.*, 1992; Kaul *et al.*, 1993: Anand and Chandrashikhar, 1995). Therefore, the depression in PLC activity observed in the present study could be due to oxidant induced alteration of the enzyme thiol group.

Other abnormalities that may also contribute towards the depression of PLC is the down-regulation in the transcription of PLC genes and changes in

lipid microdomain. Phospholipids are evenly distributed throughout the membrane, and this finding has lead to the concept of lipid micromovement or domains within the membrane, which may be due to membrane proteins having specific lipid affinities for both structural integrity and normal functioning (Panagia *et al.*, 1987, Marsh, 1992*)*. Thus, any alterations of the lipid micromovement of a given protein may induce protein dysfunction. Indeed, the alterations in SL PLC activity in CHF may arise from such changes in the membrane composition. The downregulation in the sarcolemmal PLC activity indicate the inactivation of sarcolemmal PLC enzyme and it also suggests unavailability of second messengers such as DAG and $InsP_3$ to mediate inotropic responses (Meij *et al.*, 1989; Kurz *et al.*, 1993).

The phosphoinositide specific PLC activity was also determined in the cytosolic fractions from left ventricular tissue of rat hearts at a moderate stage of congestive heart failure. No differences in the cytosolic PLC were observed in MI compared to sham control. A possible explanation of the difference observed in SL and in the cytosol may be because of the presence of different isoenzymes of PLC within these subcellular compartments. The most abundant isoform present in the sarcolemma is PLC δ-1 and in the cytosol it is PLC γ-1, which is activated by growth factor receptor kinase (Rhee *et al.*, 1991; Cockroft and Thomas, 1992; Wolf, 1992). Therefore, from the data obtained in the

current study, it can postulated that effect of imidapril may be specific for PLC δ-1 with little or no effect on PLC γ-1. The differential behaviour of PLC in the sarcolemma and cytosol at progressive stages of CHF indicates that it might be playing a distinct role in myocardial function. It would be interesting to determine PLC δ-1 (major isoform of SL) activity in the sarcolemma of imidapril treated MI animals. Also, the fact that in MI cytosolic PLC activity remains unchanged suggests that cytosolic activity is preserved in this pathological state indicating that SL phospholipase C may be more susceptible to alterations in activity in CHF.

The treatment with imidapril was started after 4 week because treatment is less effective if given near the time of infarction and specifically if there is presence of ongoing ischemia (Ball,1993). The phosphoinositide phospholipase C activity in week 8 animals after 4 week of imidapril treatment showed a partially recovery in sarcolemma. An investigation into the effect of long term treatment of ACE-inhibitor, and phosphoinositide phospholipase C after 16 week the chronic CHF should be studied. Similarly, the effect of long and short term ACE-inhibitor therapy on human myocardium should yield meaningful information.

The treatment with ACE-inhibitors has many benefits. First it reduces mortality and morbidity in patients. Second, the limitation of adverse ventricular

remodelling. Third, and most important, is the prevention of recurrent infarction. These benefits might apply to all patients after infarction, irrespective of ventricular function. These benefits might indicate a need for universal treatment (Lindsay *et al.*, 1995). Data in this study indicate that imidapril may have beneficial effect on the contractile performance of heart via increased phosphoinositide specific PLC activity. It also appears that this effect may be specific for sarcolemmal associated PLC activity.

VI. CONCLUSIONS

PI-PLC activity was examined in rats with experimentally induced MI and age matched sham control animals at postinfarction periods of 1, 2, 4, and 8 weeks in the cardiac sarcolemma and cytosolic fractions of rat heart. Imidapril treatment was administered to animals for four weeks from the beginning of 5^{th} till the end of the 8^{th} week and PI-PLC activity was determined. The following conclusions can be drawn from this study.

1. Sarcolemmal PI-PLC activity showed an 8% depression at 1 week which was not significant, however, at 2, 4, and 8 weeks a significant depression of 17% , 19% , 23% ($P<0.05$) respectively was observed as compared to values from sham control animals.
2. The PI-PLC activity in sarcolemmal membrane from experimental animal upon imidapril treatment showed a significant but partial (9%,) recovery ($P<0.05$) in MI compared to untreated MI.
3. In contrast, no significant changes were observed in the cytosolic PI-PLC activity at 1, 2, and 4 , and 8 weeks .
4. The effect of MI and imidapril on the above changes appears to be sarcolemma specific.

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