Chapter 11
Phospholipid-Mediated Signaling and Heart Disease

Paramjit S. Tappia and Tushi Singal

Abstract Cardiac hypertrophy, congestive heart failure, diabetic cardiomyopathy and myocardial ischemia-reperfusion injury are associated with a disturbance in cardiac sarcolemmal membrane phospholipid homeostasis. The contribution of the different phospholipases and their related signaling mechanisms to altered function of the diseased myocardium is not completely understood. Resolution of this issue is essential for both the understanding of the pathophysiology of heart disease and for determining if components of the phospholipid signaling pathways could serve as appropriate therapeutic targets. This review provides an outline of the role of phospholipase A_2, C and D and subsequent signal transduction mechanisms in different cardiac pathologies with a discussion of their potential as targets for drug development for the prevention/treatment of heart disease.

Keywords Cardiac hypertrophy · congestive heart failure · diabetic cardiomyopathy · ischemia-reperfusion · phospholipases

11.1 Introduction

Phospholipases play an important role in cellular metabolism, including the biosynthesis and degradation of membrane lipids. This leads to the production of many types of lipidic second messengers that mediate changes in the function of important intracellular proteins as well as the signaling of nuclear transcription factors and subsequent gene expression (Lamers et al., 1992; Dhalla et al., 2006; Tappia et al., 2006). The cardiac sarcolemmal (SL) membrane phospholipids serve as substrates for 3 major phospholipase families, phospholipase A_2 (PLA_2), phospholipase C (PLC) and phospholipase D (PLD), which produce important lipid signaling molecules (Buckland and Wilton, 2000) and have been

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localized to the SL membrane. These phospholipase enzymes are defined and named by the site of action on the phospholipid structure and thus differ in their catalytic and regulatory properties. It is pointed out that other cellular membranes such as mitochondrial, sarcoplasmic reticulum and nuclear are also important sites for the localization and action of phospholipases and thus may also play an important role in the regulation of heart function (Panagia et al., 1991; Williams et al., 1997; Cocco et al., 2006; McHowat and Creer, 2004); however, this review will largely focus on the significance of the phospholipases localized to the cardiac SL membrane. The PLA_2 isozymes represent a large family of distinct enzymes, each of which demonstrates unique characteristics (Diaz and Arm, 2003). At least four different PLA_2 isozymes exist in mammalian cells (Six and Dennis, 2000; Tanaka et al., 2000; Ohto et al., 2005). Secretory PLA_2 ($sPLA_2$), also called group II PLA_2, requires millimolar Ca^{2+} concentrations for activity, and is secreted into the extracellular space. The cytosolic PLA_2 ($cPLA_2$) also known as group IV PLA_2, requires increases in intracellular Ca^{2+} for phosphorylation of the enzyme and translocation to intracellular membrane, but does not require Ca^{2+} for its catalytic activity. The Ca^{2+}-independent PLA_2 ($iPLA_2$) or group VI PLA_2, does not require Ca^{2+} for activity. Another isoform of PLA_2 is platelet activating factor acetylhydrolase, also known as lipoprotein-associated PLA_2 (Lp- PLA_2), is a calcium-independent enzyme that cleaves oxidized and polar phospholipids (Allison et al., 2007). The expression of PLA_2 is regulated at the transcriptional level by mediators such as cytokines and growth factors, including interferon-γ, macrophage stimulating factor, tumor necrosis factor and epidermal growth factor (Hirabayashi et al., 2004). The PLA_2 enzyme activity is also enhanced by phosphorylation, a process mediated by mitogen activated protein kinases, as well as indirect activation by protein kinase C (PKC) and G-protein coupled receptors (GPCR) (Stahelin et al., 2003). To date, $sPLA_2$, $cPLA_2$ and $iPLA_2$ isozymes have been localized to the cardiac SL membrane whereas Lp-PLA_2 is bound predominantly to low density lipoprotein cholesterol (Allison et al., 2007).

The cardiac SL membrane associated PLC isozymes play a central role in activating intracellular signal transduction pathways, especially during early key events in the regulation of various cell functions (Rhee, 2001). A number of different agonists including norepinephrine (NE) and angiotensin II (ANG II), which are released by ischemic myocardial cells, bind to their respective receptors on the cell surface resulting in G-protein (Gq subfamily) activation, which can lead to subsequent stimulation of PLC (Rhee, 2001). The activation of PLC results in the hydrolysis of phosphatidylinositol 4,5 bisphosphate (PIP_2) to produce diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). While IP_3 may serve to enhance the sarcoplasmic reticulum (SR) Ca^{2+} release, DAG functions as a potent activator of most PKC isozymes, which in turn phosphorylate several cardiac proteins and stimulate Ca^{2+}-influx (Malhotra et al., 2001b; Kamp and Hell, 2000). Recently, the phenylephrine-induced transient increase in intracellular Ca^{2+}-concentration caused by Ca^{2+}-release

from SR has been demonstrated to result in a transient suppression of L-type Ca^{2+} current ($I_{Ca\text{-}L}$) in rat ventricular cardiomyocytes (Zhang et al., 2005). Interestingly, this response is followed by a potentiation of $I_{Ca\text{-}L}$ that may involve PKC. In addition, this biphasic modulation of $I_{Ca\text{-}L}$ by phenylephrine can be blocked by prazosin, indicating that these responses were mediated by the GPCR, α_1-adrenoceptor, and thus implicating a role for PLC. The PLC family consists of 6 subfamilies: PLC β, γ, δ, ε, ζ and η (Rhee, 2001; Rebecchi and Pentyala, 2000; Song et al., 2001; Saunders et al., 2002; Wing et al., 2003; Hwang et al., 2005) and are activated by different mechanisms (Lee et al., 1994; Rhee and Bae, 1997; Katan, 1998; Yagisawa et al., 1998; Fukami, 2002; Lopez et al., 2001; Yin et al., 2003). PLC β_1, δ_1, γ_1 and two forms of ε are the predominant forms expressed in the heart (Tappia et al., 1999; Lopez et al., 2001). ANG II, α_1-adrenergic agonists and endothelin-1 are relevant stimulants of PLC β isozymes via the α subunits of the heterotrimeric Gq subfamily; PLC β has also been shown to be activated by Gβγ dimer (Lee et al., 1994). A non-tyrosine kinase activation of PLC γ isozymes has been reported (Rhee and Bae, 1997), furthermore activation of PLC γ isozymes independent of tyrosine kinase has also been reported (Sekiya et al., 1999). The receptor initiated events for the activation of PLC δ isozymes are considered to be mediated via transglutaminase II, G_h, a new class of GTP binding protein (Im et al., 1997; Park et al., 2001). Although the PLC δ-G_h pathway may be an important player in the signaling pathway that regulates calcium homeostasis and modulates physiological processes. PLC ε isozymes are activated by Ras, Rho and Rap 2B as well as by $G\alpha_{12}$ (Song et al., 2001). The activation of PLC ζ and η is far less characterized.

The PLD isozymes hydrolyze phosphatidylcholine (PC) to produce PA, which is considered to be an important lipid signaling molecule. PA can be dephosphorylated to DAG by the action of phosphatidate phosphohydrolase (PAP). Thus, both PLD and PAP can modulate the levels of both PA and PLD-derived DAG in the heart. Different agents such as norepinephrine (NE), endothelin-1 and angiotensin 11 (ANG II) have been shown to increase the formation of PA in the cardiomyocytes (Sadoshima and Izumo, 1993; Ye et al., 1994). The importance of PA in heart function is evident from its ability to stimulate SL and SR Ca^{2+}-related transport systems (Dhalla et al., 1997; Xu et al., 1996b) and to increase the intracellular Ca^{2+} concentration in adult cardiomyocytes as well as augment cardiac contractile activity in the normal heart (Xu et al., 1996b). On the other hand, the *in vivo* significance of the PLD-derived DAG remains to be defined (Lamers et al., 1995; Martin et al., 1997; Hodgkin et al., 1998). Two mammalian PLD isozymes, PLD1 and PLD2, have been cloned and have ~50% identity and have been shown to be differentially regulated (Colley et al., 1997; Frohman and Morris, 1999). PLD1, which exhibits low basal activity, requires PIP_2 for its activity, and is activated by PKC and Rho small G-proteins family members (Yamazaki et al., 1999). PLD1 is localized to perinuclear regions such as endoplasmic reticulum, golgi apparatus, and endosomes. PLD2 is constitutively active and is the major SL PLD isozyme

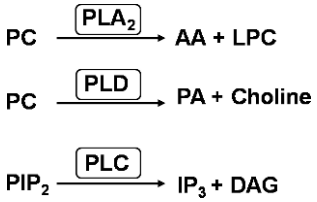

Fig. 11.1 Products of phospholipase hydrolytic activity on phospholipid substrate. PC, phosphatidylcholine; AA, arachidonic acid; LPC, lysophosphatidic acid; PA, phosphatidic acid: PIP_2, phosphatidylinositol 4,5-bisphosphate; DAG, diacylglycerol; IP_3, inositol 1,4,5-trisphosphate; PLA_2, Phospholipase A_2; PLC, Phospholipase C; PLD, Phospholipase D

in the myocardium (Park et al., 2000). PLD2 also requires PIP_2 for its activity, but unlike PLD1, it is activated by unsaturated fatty acids such as AA and oleate (Dai et al., 1995; Liu et al., 1998) and is insensitive to the PLD1 activating factors (Colley et al., 1997). Figure 11.1 illustrates the hydrolysis of membrane phospholipids by 3 major phospholipases resulting in the production of intracellular second messengers that may lead to altered signaling in the heart.

11.2 Cardiac Hypertrophy is Associated with Activation of Different Phospholipases

The American Heart Association Statistics Committee, 2008 has reported heart disease to be the leading cause of death in the western world. Cardiovascular disease claims one life every 33 seconds (Rosamond et al., 2008). In the year 2007, 15.8 million Americans suffer from cardiovascular disease and 0.45 million died of it and 1234 Americans die everyday (National Centre for Health Statistics). Pathological or reactive cardiac growth is triggered by autocrine and paracrine neurohormonal factors released during biomechanical stress that signal through the Gq/PLC pathway, leading to an increase in cytosolic Ca^{2+} and activation of PKC (Dorn and Force, 2005). In this regard, in stroke prone spontaneously hypertensive rats, the development of cardiac hypertrophy have been suggested to involve an increase in PLC signaling pathway (Kawaguchi et al., 1993). PLC ε-deficient mouse strain had decreased cardiac function and decreased contractile response to acute isoproterenol administration (Wang et al., 2005). These studies suggested that loss of PLC ε signaling sensitizes the heart to development of hypertrophy during chronic cardiac stress. Studies with the cardiomyopathic hamster (BIO 14.6) have shown that cardiac hypertrophy is due to increase in PLC activity as a consequence of enhanced responsiveness to ANG II (Sakata, 1993); in fact, ANG II can initiate cardiac hypertrophy and upregulate signal molecules in the Gαq/11-mediated signal transduction pathway, such as PLC β_3, and ERK1/2, at both tissue and cellular levels (Bai et al., 2004). Cardiac hypertrophy due to volume overload induced by arteriovenous

(AV) shunt has been linked to PLC β_1 and γ_1 activation (Dent et al., 2004a). Furthermore, increases in specific PLC isozyme mRNA levels has also been observed in atrial and right ventricular hypertrophy due to volume overload (Dent et al., 2006). It is interesting to note that pressure overload induced cardiac hypertrophy in guinea pigs, due to a ligature around the descending thoracic aorta, is associated with an increase in PLC β_1 activity (Jalili et al., 1999). Fas receptor activation is an important component in hypertrophy induced by pressure- and volume-overload and recently it has been reported that Fas-mediated hypertrophy is dependent on the IP_3 pathway, which is functionally inter-connected to the PI3K/AKT/GSK3β pathway; both pathways act in concert to cause NFAT nuclear translocation and subsequent hypertrophy (Barac et al., 2005). Recent evidence suggests that ventricular pressure overload hypertrophy led to an upregulation of PLD isozymes both in rat and human heart which may be due to potentiation of PLD activation by α-adrenoceptor and PKC stimulation (Peivandi et al., 2005). PA produced by the activation of PLD also stimulates SL PLC activity (Dhalla et al., 1997; Tappia et al., 2001b). Since DAG, formed due to the activation of PLC, is considered to play a crucial role in regulating the activity of PKC, the positive feedback effect of PA on this pathway may be essential for maintaining the sustained elevation in the activity of PKC during the development of cardiac hypertrophy.

Stimulation of signaling pathways via Gqα provokes cardiac hypertrophy in cultured cardiomyocytes and transgenic mouse models (D'Angelo et al., 1997; Sakata et al., 1998). ANG II receptor, type 1 overexpression has been reported to induce cardiac hypertrophy (Paradis et al., 2000). In this regard, it is pointed out that an essential downstream effector for Gqα is PLC β (Rhee, 2001). The first transgenic murine cardiac hypertrophy model to support a Gqα mechanism of hypertrophy was over expression of the constitutively activated Gq coupled to α_{iA} adrenergic receptor (Milano et al., 1994). In these hearts a chronic activation of PLC resulted in hypertrophy and an increase in the hypertrophic marker gene atrial natriuretic factor (ANF). In isolated adult left ventricular cardiomyocytes we have reported that the NE-induced increases in ANF gene expression and protein synthesis that can be attenuated by a PLC inhibitor, U73122, as well as by an α_1-adrenoceptor blocker, prazosin (Singal et al., 2004). The receptor of most growth factors are transmembrane tyrosine kinases and transduce their signal via PLC γ, thus implicating this family of PLC isozymes in cardiac hypertrophy (Hefti et al., 1997). We have recently established that PLC activities regulate their own isozyme gene expression in a PKC-ERK1/2-dependent pathway, which may represent a cycle of events associated with the cardiomyocyte hypertrophic response to NE (Singal et al., 2006). The development of hypertrophy in cultured rat neonatal cardiomyocytes induced by endothelin-1 has been reported to be due to activation of PLC β isozymes (Lamers et al., 1995). In addition, recent studies in neonatal rat cardiomyocytes stimulated with different hypertrophic stimuli, have shown an increased mRNA expression and protein level of PLC β isozymes (Schnabel

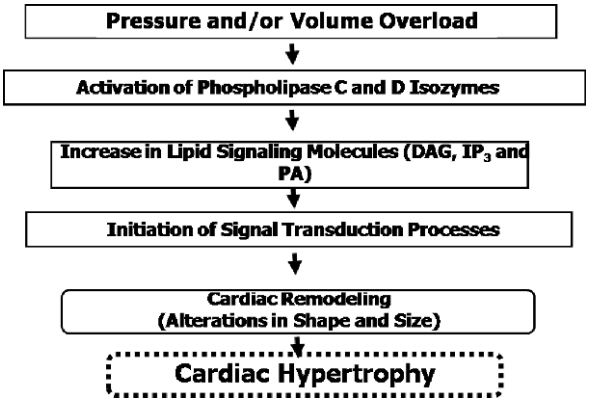

Fig. 11.2 Hemodynamic overload leading to cardiac hypertrophy through activation of Phospholipase C and D. DAG, diacylglycerol; IP_3, inositol 1,4,5-trisphosphate; PA, phosphatidic acid

et al., 2000). In brief, the above evidences suggest that activation of phospholipases play a key role in mediating cardiac hypertrophy as shown in Fig. 11.2.

11.3 Differential Changes in Phospholipases in Congestive Heart Failure

In 2004, 1 in 8 death certificates (284,365 deaths) in the US indicates heart failure as the cause of death with total death rate for heart failure were 52 per 1000. Total death rate was 63.2 for white males, 43.5 for white females and 78.8 for black males and 58.7 for black females. The estimated direct and indirect cost of heart failure in the US for 2008 is 34.8 billion (Heart disease and stroke statistics-2008 update, American Heart Association, Rosamond et al., 2008). Congestive heart failure (CHF) is invariably preceded by cardiac hypertrophy as an adaptive mechanism due to a wide variety of neurohumoral changes; however, the mechanisms for the transition of cardiac hypertrophy to heart failure are poorly understood. Although the initial outcome is a compensatory growth of the heart, prolonged development of hypertrophy leads to CHF. We have reported that abnormalities in protein abundance and activity and in the cellular localization of myocardial PLA_2 isozymes occur at the overt stage of CHF after myocardial infarction (MI) (McHowat et al., 2001). Since PLD-derived PA influences intracellular Ca^{2+} concentration and contractile performance of the cardiomyocytes, changes in $iPLA_2$ activity may contribute to abnormal contractile performance of the failing heart via an impaired interaction of PLA_2 with PLD pathway.

We have examined the changes in activities and SL protein abundance of PLC isozymes in CHF due to MI (Tappia et al., 1999, 2001b; Ju et al., 1998).

While profound decreases in SL PLC γ_1 and δ_1 activities and protein levels occur in CHF, treatment of animals with the ACE inhibitor imidapril partially corrected these changes in PLC isozymes forms as well as cardiac function (Tappia et al., 1999). This would suggest that RAS may be involved in mediating alterations in PLC isozymes and that PLC isozymes could serve as novel targets for the treatment of CHF. In addition, PLC isozymes could be another target for the mechanisms of action of ACE inhibitors. It is pointed out that an upregulation of the Gqα/PLC-β pathway in the viable, border, and scar tissues in the post-MI hearts is also seen, which may play an important role in cardiac fibrosis and scar remodeling (Ju et al., 1998).

Differential changes in SL PLC isozyme activities and their SL abundance in the failing heart of the cardiomyopathic hamster (UM X7.1) have also been detected (Ziegelhoffer et al., 2001). Although a decrease in PLC δ_1 isozyme activity and an increase in PLC β_1 and γ_1 isozyme activities were detected, an elevation of IP_3 levels were seen; while the relevance of the changes of PLC isozymes remains to be defined, the increase in IP_3 may contribute to intracellular calcium overload in the failing cardiomyocytes of cardiomyopathic hamster. Volume overload induced cardiac hypertrophy invariably precedes CHF and in this model we have also reported decreases in PLC isozymes and SL level of PIP_2 (Dent et al., 2004b). It is interesting to note that in the cardiomyopathic hamster and in CHF due myocardial infarction and volume overload the decrease in SL PLC δ_1 activity correlates to its reduced SL protein abundance (Dent et al., 2004a; Ziegelhoffer et al., 2001). The NH_2-terminal part of the PH domain of PLC δ_1 has a high affinity for the polar head group of PIP_2 that confers a unique capacity of PLC δ_1 to associate with the SL membrane (Tall et al., 1997). The reported decrease in the SL PIP_2 content may be a mechanism, which reduces the attachment of PLC δ_1 to the SL membrane. It should be noted that in addition to PIP_2 serving as a substrate for PLC and as a membrane attachment site, a number of diverse biochemical events are also regulated by PIP_2 and are affected by the altered concentration of this lipid in the membrane. Of note, the decreased number of PIP_2 molecules could compromise the contractile performance of the heart by directly causing a depression of the inward rectifier K^+ channels (Huang et al., 1998), as well as depression of the SL Na^+-Ca^{2+} exchanger and Ca^{2+}-pump activities (Hilgemann and Ball, 1996). Therefore, a diminished amount of PIP_2 inside the SL membrane may be critical for cardiac dysfunction during CHF.

Although in CHF due to MI an increase in PLD2 gene expression as well as SL PLD2 protein abundance and activity has been reported, an increase in PLD2 protein and activity has also been detected in scar tissue that may be involved in scar remodeling (Dent et al., 2004b). In addition, a greater increase in SL phosphatidate phosphohydrolase (PAP) type 2 activity was observed; the net effect of PLD-derived PA formation and PAP-mediated dephosphorylation of PA was a decrease in the SL PA level and an impairment of the bioprocesses mediated by SL PA as well as a defective interaction between SL PLD and PLC signaling pathways as evidenced by a loss of PA-induced increase in Ca^{2+}

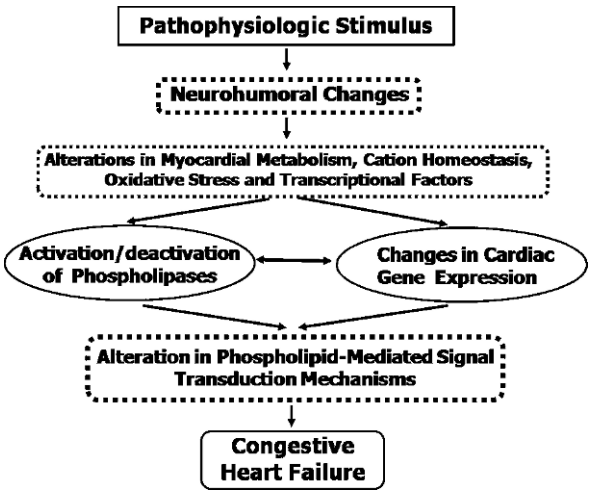

Fig. 11.3 Mechanisms contributing to altered phospholipid-mediated signal transduction in congestive heart failure

transients due to a diminished stimulation of PLC activities in failing cardiomyocytes (Tappia et al., 2001b, 2003). This signal transduction pathway could constitute an important target for pharmacological interventions as treatment of CHF animals with imidapril, an angiotensin converting enzyme (ACE) inhibitor, normalized PA levels in the cardiac SL membrane (Yu et al., 2002). Figure 11.3 depicts the possible mechanisms that lead to alterations in the different phospholipase isozyme activities and subsequent changes in phospholipid-mediated signal transduction mechanisms leading to congestive heart failure. In summary, it is evident that phospholipid-signaling systems may have an important role in cardiac hypertrophy as well as contractile dysfunction in CHF and may be potential targets for drug development.

11.4 Phospholipid-Mediated Signaling in Diabetic Cardiomyopathy

The World Health Organization estimates that by 2015, the number of overweight people globally will increase to 2.3 billion, and more than 700 million people will be obese. Mayo clinic data indicate that the prevalence of diabetes increased 3.8% every year. The total prevalence of diabetes mellitus in US is expected to more than double from 2005 to 2050 in all age, sex and race groups. Data from the National Diabetes Information Clearing House, states at least 65% of people with diabetes die of some form of heart disease or stroke. Among women with CHD, diabetes is the strongest risk factor for heart failure in US (American Heart Association, 2008, Rosamond et al., 2008). In 2002, the direct

and indirect cost attributable to diabetes was $132 billion. A disproportionately high prevalence of diabetes in African and Mexican Americans when compared to Caucasians has been observed. Cardiovascular disease is responsible for 80% of deaths among diabetic patients much of which has been attributed to coronary artery disease (Hayat et al., 2004). In fact, the incidence of heart disease is greater in the diabetic population than the non-diabetic population (Regan, 1983). However, the presence of a primary cardiomyopathy in diabetes has been long identified (Dhalla et al., 1985; Fein et al., 1980; Fein and Sonnenblick, 1985; Penpargkul et al., 1980; Regan et al., 1974). Diabetic cardiomyopathy is a cardiac disease that arises as a result of the diabetic state, independent of vascular or valvular pathology. It manifests initially as asymptomatic diastolic dysfunction, which progresses to symptomatic heart failure. The compliance of the heart wall is decreased and contractile function is impaired. The pathophysiology is incompletely understood, but appears to be initiated both by hyperglycemia and changes in cardiac metabolism. These changes induce oxidative stress and activate a number of secondary messenger pathways, leading to cardiac hypertrophy, fibrosis and cell death (Sharma and McNeill, 2006). A number of animal studies have found that this cardiomyopathic condition is associated with defects in the capacity of cardiomyocytes to regulate intracellular ionic homeostasis in a normal manner (Allo et al., 1991) resulting in abnormal Ca^{2+} transients and contractile activity (Lagadic-Gossmann et al., 1996; Ganguly et al., 1983; Heyliger et al., 1987; Horackova and Murphy, 1988). Furthermore, alterations in contractile proteins and intracellular ions impair excitation-contraction coupling, while decreased autonomic responsiveness and autonomic neuropathy impair its regulation. Extensive structural abnormalities also occur, which have deleterious mechanical and functional consequences (Sharma and McNeill, 2006). The overpresentation of diabetic patients with heart failure trials such as SOLVD (Studies Of Left Ventricular Dysfunction); 26% (Shindler et al., 1996), ATLAS (Assessment Trial of Lisinopril And Survival); 19% (Ryden et al., 2000) and V-HeFT II (Vasodilator-Heart Failure Trial II); 20% (Cohn et al., 1991) attests to the prevalence of this condition in the diabetic population.

Diabetic cardiomyopathy is a result of maladaptive changes in energy homeostasis. Although diabetes-induced changes in PLA_2 activities have been measured in several tissues, very little is known about the status of PLA_2 isozymes in diabetic myocardium. An increased membrane-associated $iPLA_2$ activity however, has been observed in the hearts of rats with streptozotocin (STZ)-induced diabetes (McHowat et al., 2000), which may be due to the diabetes-induced increase in $iPLA_2$ mRNA level in rat myocardium (Su et al., 2005). This increase in $iPLA_2$ activity was accompanied by an increase in LPC production. These investigators also demonstrated that the diabetes-induced changes in $iPLA_2$ activity and LPC production were reversed by insulin treatment of diabetic animals and concluded that diabetes-induced changes in membrane phospholipid content and phospholipid hydrolysis may contribute to some of the alterations in myocardial function that are observed in diabetic patients. In

addition, the molecular species of the major phospholipid classes in SL membrane of STZ-diabetic rats have also been examined (Vecchini et al., 2000). The relative content of plasmalogens increased in all the phospholipid classes of diabetic SL membrane. PC and phosphatidylethanolamine were mostly enriched with molecular species containing linoleic acid and deprived of the molecular species containing AA. The molecular species of phosphatidylserine containing either AA or docosahexaenoic acid were less abundant in membranes from diabetic rats than in membranes from controls. Insulin treatment of diabetic rats restored the species profile of phosphatidylethanolamine and overcorrected the changes in molecular species of PC. These investigators concluded that the high SL level of plasmalogens and the abnormal molecular species of glycerophospholipids may be critical for the membrane dysfunction and defective contractility of the diabetic heart.

While ANG II and PKC have been implicated in cardiac dysfunction during diabetes (Malhotra et al., 2001a; Liu et al., 1999), virtually nothing is known about the status of PLC in the diabetic heart. In acute diabetes (3 days, after the induction) the enhanced inotropic response to methoxamine, an α_1-adrenoceptor agonist, was ascribed to an increased PLC activity (Wald et al., 1988). We have earlier reported that the total cardiac SL PLC activities are significantly decreased in STZ-induced chronic diabetic rats under *in vitro* assay conditions (Tappia et al., 2001a). In isolated cardiomyocytes we also observed a reduced concentration of basal as well as PA-induced IP_3 generation in diabetic rats (Tappia et al., 2004b), suggesting that decreased basal PLC activities *in vivo* may exist in diabetic cardiomyopathy. We have recently reported that the decrease in the total SL PLC in diabetes is associated with a decrease in SL PLC β_3 activity, which immunofluorescence in frozen diabetic left ventricular tissue sections revealed to be due to a decrease in PLC β_3 protein abundance; a 2-week insulin treatment of 6 wk diabetic animals partially normalized these parameters (Tappia et al., 2004a). The functional significance of the defective total PLC activities, including PLC β_3 activity, and diminished levels of IP_3, is that it may constitute a mechanism for the reported reduced force of contraction in response to α_1-adrenergic stimulation of the isolated papillary muscle (Heyliger et al., 1982), however an enhanced inotropic response to α_1-adrenergic stimulation in the isolated working heart from diabetic rats has also been reported (Heijnis et al., 1992). On the other hand, a reduced production of PLC-derived DAG would affect several cellular processes (Puceat and Vassort et al., 1996). While abnormalities in other signaling pathways occur during diabetes, in particular, the β-adrenoceptor induced increases in contractions and $[Ca^{2+}]_i$ transients which are markedly diminished (Tamada et al., 1998; Ha et al., 1999), it can be suggested that an impairment of PLC signaling mechanisms may also significantly contribute to a defective cardiac contractile performance during diabetes. Furthermore, it is pointed out that depressed activities of other PLC isozymes in diabetic cardiomyopathy have also been observed (Tong et al., 1998; Tappia et al., 2000).

Although the positive inotropic effect of PA on the isolated perfused heart of STZ and alloxan-induced diabetic rats has been demonstrated (Xu et al., 1996a), the effects of exogenous PA on Ca^{2+} transients and contractile activity have also been reported in cardiomyocytes isolated from chronic STZ-induced diabetic rats. The PA induced contractility was correlated to an attenuated PA-induced IP_3 generation in diabetic rat cardiomyocytes (Tappia et al., 2004b). Insulin treatment of the diabetic animals resulted in a partial recovery of PA responses and it was suggested that a defect in the PA-PLC signaling pathway in diabetic rat cardiomyocytes may contribute to the depressed cardiac contractile performance during diabetes, similar to the defect in CHF (Tappia et al., 2003). A number of possible mechanisms can be proposed to explain the depressed PLC activities in diabetic cardiomyopathy. As already indicated PA is a potent stimulator of PLC. A decrease in SL PA formation due to an impaired PLD activity has been reported (Williams et al., 1998), which could result in an attenuated stimulation of PLC. In this regard, it is interesting to note that the marked reduction of AA content of PC in SL membrane of diabetic heart could represent a mechanism of a defective PLD activity. An increase in total myocardial DAG level has been reported in STZ-induced diabetic rats and in spontaneous autoimmune diabetic BB rats (Okumura et al., 1988; Inoguchi et al., 1992). Increase in membrane DAG content has been shown to destabilize the membrane and structural transitions (Das and Rand, 1984; Das and Rand, 1986) and this may have an inhibitory effect on PLC activity. It is pointed out that oxidative stress has been shown to occur during diabetic cardiomyopathy (Dhalla et al., 1998). Since SL PLC is inhibited by oxidants through reversible modification of the associated thiol groups (Meij et al., 1994), the depressed PLC activities seen in diabetes could also in part be explained by the oxidant-induced alteration of thiol groups. *In vitro* studies have demonstrated that LPC inhibits both SL PI 4 kinase and PI 4-P 5 kinase activities (Liu et al., 1997). This is of particular relevance as LPC accumulates in SL during diabetic cardiomyopathy, suggestive of a diminished synthesis of PIP_2 substrate for PLC. Furthermore, oxidants have also been shown to inhibit both SL PI 4 kinase and PI 4-P 5 kinase activities (Mesaeli et al., 2000), as well as SL total PLC activity (Meij et al., 1994). Since substrate availability determines hydrolytic activity of PLC, such mechanisms could additionally contribute to a decrease in PLC activity. In addition, the decrease SL PIP_2 level may also contribute to the depressed cardiac contractility independent of the effects on PLC activities (Huang et al., 1998). It is also interesting to note that the hexosamine pathway has been suggested to inhibit phenylephrine-induced inotropy of the diabetic heart (Pang et al., 2004), which may be related to defective PLC activities. In summary, on the basis of the limited information available in the literature, it can be suggested that diabetes-induced changes in the membrane composition as well as phospholipid-mediated signaling systems may contribute to the depressed contractility of the diabetic myocardium (Fig. 11.4).

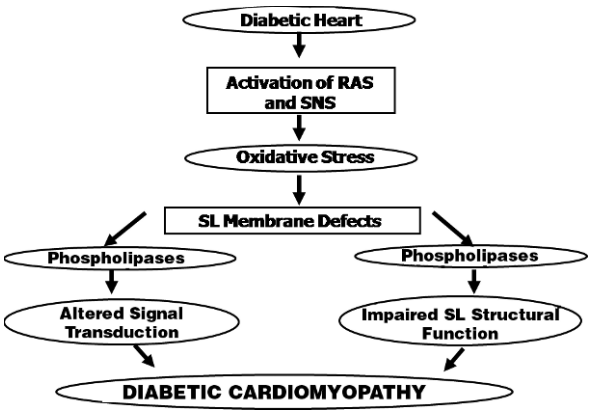

Fig. 11.4 Diabetes-induced generation of free radicals leading to altered membrane composition and sarcolemmal phospholipid-mediated signaling systems. RAS, renin angiotensin system; SNS, sympathetic nervous system; SL, sarcolemma

11.5 Myocardial Ischemia-Reperfusion and Alterations in Phospholipid-Mediated Signal Transduction

Approximately 12 million visited a physician's office for ischemic heart disease (IHD) in the USA 2001 (National Hospital Ambulatory Medical Care Survey: 2001). Presently, it is estimated that 18.5 million people in the USA suffer from IHD. Of particular concern are the growing disparities in IHD mortality among African Americans relative to Hispanics and non-Hispanic whites (Karter et al., 1998). Myocardial ischemia is known to produce dramatic changes in cardiac function, metabolism and ultrastructure (Jennings and Reimer, 1991; Hearse and Bolli, 1992) as well as proteolysis (Yoshida et al., 1995), DNA fragmentation (Scarabelli et al., 2001) and oxidative stress (Dhalla et al., 1999; Dhalla et al., 2000) (Fig. 11.5); however, the cellular and molecular events leading to contractile dysfunction and derangement of cardiac structure are not clearly understood. Although re-institution of coronary flow to the ischemic heart is considered beneficial for the recovery of cardiac pump function, reperfusion after a certain period of ischemia has been shown to further aggravate the myocardial abnormalities (Bolli and Marban, 1999; Dhalla et al., 1999, 2000; Piper et al., 2003; Marczin et al., 2003; Kim et al., 2003). Ischemia-reperfusion (I-R) injury is known to occur during, clinical procedures such as coronary bypass surgery, angioplasty, thrombolytic therapy and cardiac transplantation (Dhalla et al., 1999). The cardiac pump failure and changes in cardiac cell ultrastructure due to I-R or hypoxia-reoxygenation involve a variety of complex pathophysiological abnormalities and our current information on these aspects is largely based on the beneficial effects of a number of drug interventions for the treatment of IHD. For example, the beneficial effects of Ca^{2+} antagonists (Urquhart et al., 1985; Cavero and Spedding, 1983) and Na^{+}-H^{+} exchange

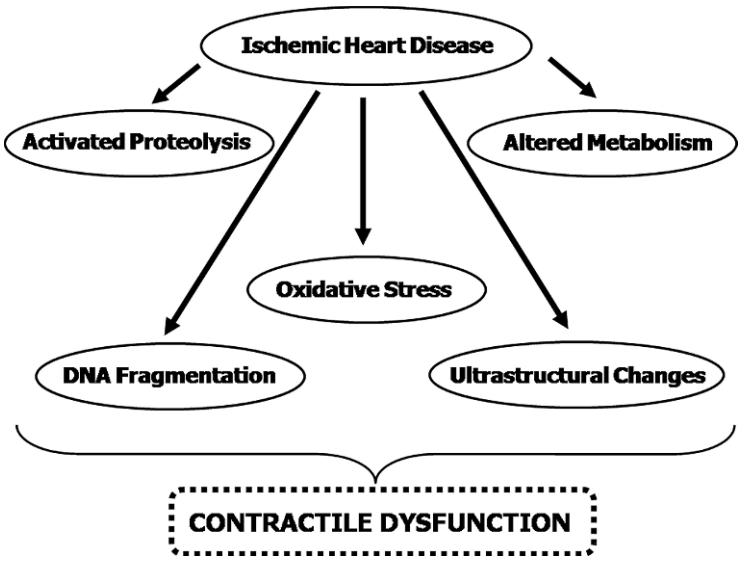

Fig. 11.5 Cardiac and other defects associated with ischemic heart disease

inhibitors (Avkiran and Snabaitiset al, 1999; Hartmann et al., 1999; Haist et al., 2003; Xiao et al., 2003) have supported a role of intracellular Ca^{2+}-overload (Dhalla et al., 2001; Miyamae et al., 1996; Jeremy et al., 1992), whereas those of antioxidants (Dhalla et al., 1999, 2000) suggest the involvement of oxidative stress in the pathophysiology of IHD. Indeed, the role of reactive oxygen species (ROS) in the genesis of myocardial cell damage and subsequent contractile dysfunction is established (Kloner et al., 1983; Ungvari et al., 2005). Since intracellular Ca^{2+} overload is considered to play a crucial role in the I-R injury and cardiac dysfunction (Dhalla et al., 1978, 1982), it is possible that several mechanisms, which are involved in the regulation of Ca^{2+} movements in the myocardial cell, are altered by ROS, including phospholipases.

The activation of PLA_2 is known to lead to an accumulation of AA within the membrane phospholipid pool of the ischemic myocardium. Some metabolites of AA produce detrimental effects in the heart in ischemia and proinflammatory effects in reperfusion, whereas others have been recently shown to reduce I-R injury in the heart (Gross et al., 2005). Choline released into the heart perfusate is found to be a useful indicator of phospholipid degradation caused by I-R (Bruhl et al., 2004). Choline glycerophospholipid, in particular PC and plasmenylcholines, are degraded by PLA_2 to lysophospholipids and lyosophosphatidylcholine (LPC), which is a known arrhythmogenic agent. Recent studies have revealed that LPC produces mechanical and metabolic derangements in working rat hearts, and Ca^{2+} overload in isolated cardiomyocytes. Thus, LPC possesses an ischemia like effect on the heart. LPC accumulated in the myocardium activates PLA_2, establishing a vicious cycle of exacerbated LPC production (Hashizume et al., 1997).

Although I-R induced changes in PLC activities have been reported to be associated with cardiac dysfunction due to I-R (Anderson et al., 1995; Otani et al., 1988; Moraru et al., 1995; Kurz et al., 1999; Mouton et al., 1991; Schwertz and Halverson, 1992; Munakata et al., 2002), in these studies no attempt was made to identify specific PLC isozyme changes. We have been the first to report that while cardiac ischemia is associated with an activation of SL PLC β_1 and decreased SL PLC γ_1 and δ_1 activities, reperfusion of the ischemic heart results in activation of SL PLC γ_1 and δ_1 isozymes, whereas PLC β_1 activity progressively declines (Asemu et al., 2003, 2004). Although exposure of SL membranes and isolated cardiomyocytes to oxidants induces changes in PLC and components of the phosphoinositide pathway (Meij et al., 1994; Mesaeli et al., 2000), the effects of oxidants on specific PLC isozymes has not been completely examined. In this regard, we are also the first to have reported that treatment of cardiomyocytes with H_2O_2 results in an activation of PLC γ_1 (Mangat et al., 2006). It was suggested that PLC γ_1 might play a role in cardiomyocyte survival during oxidative stress via PKC ε and phosphorylation of Bcl-2. Furthermore, blockade of PLC activities with U73122 results in an augmentation of the H_2O_2 induced cardiomyocyte apoptosis, while no effect on H_2O_2 induced necrotic cell death (Asemu et al., 2003). These data suggest that PLC-mediated signaling transduction may initiate anti-apoptotic signals in cardiomyocytes during oxidative stress. Cardiac I-R is also associated with an increase in PLC δ_1. Given that PLC isozymes are dependent on Ca^{2+}, they activate Ca^{2+}-transporting systems and that PLC δ isozymes are considered Ca^{2+}-amplifiers (Rebecchi and Pentyala, 2000), it is conceivable that activated PLC δ_1 may contribute to a self-perpetuating cycle that exacerbates cardiomyocyte Ca^{2+}-overload and subsequent cardiac dysfunction during I-R. Although it appears that Ca^{2+} may be involved in the activation of this PLC isozyme (Asemu et al., 2004), the role of oxidants cannot be excluded. However, the mechanisms responsible for and the significance of the changes in specific SL PLC isozyme activities, protein contents and gene expression with respect to Ca^{2+}-homeostasis and cardiac dysfunction in I-R have not been investigated.

The I-R-induced changes are not limited to PLA_2 and PLC. Some studies have examined the redox regulation of the cardiac PLD activities in the setting of I-R or with isolated cardiac SL and SR membrane preparations. A recent study has suggested that a direct Rho A-PLD1 interaction stimulates PLD1 activity, which mediates the cardioprotective effect of adenosine A3 receptor, establishing an important antiischemic role of PLD (Mozzicato et al., 2004). While some investigators have reported that the activation of PLD is associated with an improvement of post-ischemic functional recovery and attenuation of cellular injury (Tosaki et al., 1997), other studies, as well as work from our laboratory have found variable changes in the PLD activity in the ischemic heart (Bruhl et al., 2003; Kurz et al., 2004; Asemu et al., 2005). Our studies have revealed that PLD2 activity is increased in early reperfusion of the 30 min ischemic heart, whereas in prolonged reperfusion PLD2 activity is significantly depressed (Asemu et al., 2005). While the activation of PLD2 may represent a cell survival

response, evoked by low concentrations of oxidants for a relatively short period of time, the depressed activity may be in response to cardiomyocyte damage due to a more prolonged exposure to high concentrations of oxidants and may be linked to the poor functional recovery of the heart due to oxidative stress following I-R. In this regard, it is known that I-R leads to depletion of GSH content (Ferrari et al., 1991; Ozer et al., 2005), which is the major intracellular non-protein sulfydryl and plays an important role in the maintenance of cellular proteins and lipids in their functional state and acts primarily to protect these important structures against the threat of oxidation (Wu et al., 2004; Hurd et al., 2005). In view of the observed depression of PLD2 activity in prolonged reperfusion (Asemu et al., 2005), it is conceivable that this may be due to oxidation of PLD2. The increase in the SL PLD2 activity in early reperfusion may be due to post-translational modifications as a result of the formation of ROS. On the other hand, we have reported that a Ca^{2+}-independent PLA_2 and subsequent mobilization of the unsaturated fatty acid has been shown to modulate the activity of PLD in heart SL (Liu et al., 1998). Interestingly, the PLA_2 is also activated by H_2O_2 (Sapirstein et al., 1996), which could provide a mechanism of an indirect regulation of the SL PLD2 activity by H_2O_2. It is pointed out that the basal myocardial PLD activity has been reported to be decreased by the tyrosine kinase inhibitor, genistein, and increased by vanadate, a tyrosine phosphatase inhibitor (Lindmar and Loffelholz, 1998). In view of the fact that tyrosine kinases are activated in response to oxidants (Snabaitis et al., 2002; Purdom and Chen, 2005), it is conceivable that SL PLD isozyme activities could also be indirectly regulated by oxidant-induced activation of tyrosine kinase activities. Since a significant degree of PLD activity is also localized in myocardial SR membranes (Panagia et al., 1991), it is possible that it may take part in the regulation of Ca^{2+}-movements (Asemu et al., 2005). Thus, we also observed a decrease in the SR PLD2 activity after 5 min of reperfusion, which was suggested to be a reversible oxidation because the SR PLD2 activity was recovered after 30 min reperfusion.

While both SL and SR PLD activities, *in vitro*, have been reported to be inhibited by oxidants such as H_2O_2 and HOCI, through reversible modification of associated thiol groups (Dai et al., 1992, 1995), some of the inconsistencies between the observations in the isolated perfused heart and oxidant effects on SR and SL preparations could be explained on the basis that the functional thiol groups of the SL PLD2 in the isolated perfused heart are not as readily accessible by oxidants as these are in the isolated SL preparation. Such responses may also be due to differences in the sensitivity of the SR and SL PLD to different concentrations of oxidant molecules as well as ROS. Nonetheless, the elucidation of the detailed mechanism of PLD activation by thiol modulating agents will be of importance for clearly understanding the oxidant-induced signal transduction pathways and PLD regulation under different types of oxidative stress.

A comment about redox signaling must also be made. Although it is well known that I-R results in cardiomyocyte death by apoptosis as well as necrosis (Olivetti et al., 1997), it has also been shown that ROS produced during I-R can induce a number of anti-apoptotic genes and transcription factors (Nishio et al., 1998).

Thus, it appears that cardiomyocyte death induced by I-R is a net effect of the redox regulated cell survival signals and ROS triggered cell death mechanisms. The first unequivocal evidence for the role of ROS as a second messenger for cell survival was observed with the production of ROS during the agonist-induced activation of NFκB which regulates the inducible expression of a number of genes such as Bcl-2 and pro-apoptotic factors including Bax and p53, in the I-R myocardium (Bromme and Holtz, 1996) via a signal transduction pathway that could involve PLD. Subsequently, myocardial adaptation to ischemia due to ischemic preconditioning (IP), where a brief period of ischemia prior to a prolonged period improves myocardial function and diminishes the infarct size, was also observed to be associated with the generation of ROS (Tritto and Ambrosio, 2001; Dhalla et al., 1998). It was indicated that PLD may play an important role in IP and may be related to ROS generation during IP (Tritto and Ambrosio, 2001; Dhalla et al., 1998). In fact, the redox signaling which is considered to protect the heart during IP involves the phosphorylation of tyrosine kinases and activation of multiple kinases (Moraru et al., 1992; Cohen et al., 1996), including PKC isozymes (Eskildsen-Helmond et al., 1996). Such a cascade of signal transduction for all survival has been linked to the activation of PLD (Moraru et al., 1992; Cohen et al., 1996; Trifan et al., 1996). It should be noted that agonists of PLD simulate the effects of IP, whereas the inhibition of PLD blocks the beneficial effects of IP (Ozer et al., 2005; Hashizume et al., 1997). In summary, the two extremes of stress imposed on the heart under I-R change the redox potential of the cardiomyocyte and affect redox-sensitive molecules involved in phospholipid-mediated signal transduction mechanisms. The early activation of specific PLC and PLD isozymes may represent initiation of redox signaling and cell survival pathways, whereas inactivation of specific PLC and PLD isozymes may contribute to impaired cardiac contractile function (Fig. 11.6).

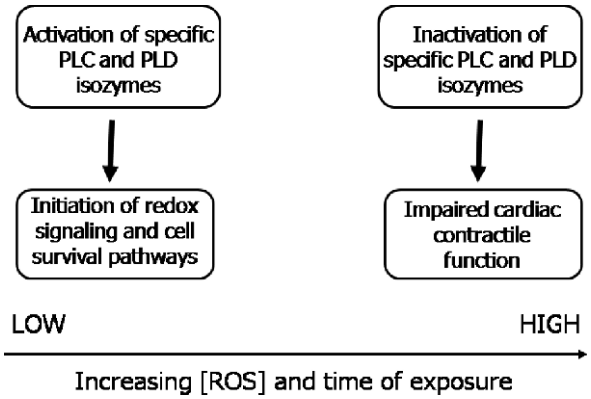

Fig. 11.6 Role of Phospholipase C and D in the cardiac response to different concentrations and time of exposure to reactive oxygen species. ROS, reactive oxygen species; PLC, Phospholipase C; PLD, Phospholipase D

11.6 Concluding Remarks

Several lines of evidence have revealed that phospholipid-mediated signal transduction mechanisms are impaired in the diseased myocardium. However, the contribution of these signaling systems with respect to other myocardial signaling systems, to cardiac function and the myocardial genetic machinery needs to be defined. The precise mechanisms of regulation of the cardiac phospholipase activities also remain to be completely understood. Although the cellular and molecular mechanisms responsible for changes in phospholipid signaling systems are being investigated, it could emerge that specific phospholipase isozymes might constitute additional therapeutic targets for drug discovery for the treatment of heart disease, however with the increasing complexity of phospholipid-mediated signal transduction mechanisms, the achievement of this outcome will be a major challenge.

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