Review

Mechanisms for the Defects in Phospholipid Signal Transduction in Diabetic Cardiomyopathy

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Although diabetic cardiomyopathy is associated with heart dysfunction and disturbance in cardiac sarcolemmal membrane phospholipid composition, the role of the different phospholipases and their related signaling mechanisms to altered function of the heart in diabetes is not completely understood. Thus, understanding the pathophysiology of cardiovascular abnormalities in diabetes, as well as identifying defects in various components of the phospholipid signaling pathways, that could serve as therapeutic targets, is warranted. Accordingly, this review provides an outline of the role of and the mechanisms for the defects in phospholipase A_2, C and D-mediated signal transduction in the diabetic heart. In addition, the potential of different phospholipases as targets for drug development for the prevention/treatment of heart disease in diabetes is discussed.

Keywords: Diabetes, Heart dysfunction, Diabetic cardiomyopathy, Phospholipases, Signal transduction.

Introduction

Phospholipases play an important role in cellular metabolism for the degradation of membrane lipids. The activation of these enzymes 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. The cardiac sarcolemmal (SL) 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 and have been localized to the cardiomyocyte membrane. These phospholipase enzymes are defined by the site of action on the phospholipid structure and thus differ in their catalytic and regulatory properties. Other cellular membranes, such as mitochondrial, sarcoplasmic reticulum (SR) and nuclear are also important sites for the localization and action of phospholipases and therefore may also play an important role in the regulation of heart function; however, this review will largely focus on the significance of the phospholipases localized to the cardiac SL membrane.

Biochemical characteristics of phospholipases

PLA_2 Isozymes

The PLA_2 isozymes are a large family of distinct lipolytic enzymes, each of which demonstrates unique characteristics that catalyze the hydrolysis of the ester bond at the sn-2 position of phospholipids to produce the free fatty acid and the lysophospholipid. PLA_2 enzymes are classified into 5 major groups with several subgroups in mammalian cells. Secretory PLA_2 (sPLA_2), also referred to as group II PLA_2, requires millimolar Ca²⁺ concentrations for activity and is secreted into the extracellular space. Another secreted isoform of PLA_2 is platelet activating factor acetylhydrolase, also known as lipoprotein-associated
PLA2 (Lp-PLA2), is a calcium-independent enzyme that cleaves oxidized and polar phospholipids and is associated with plasma lipoproteins\textsuperscript{10,14}.

The other 3 groups of PLA2 enzymes are intracellular — the cytosolic PLA2 (cPLA2) also termed as group IV PLAs requires increases in intracellular Ca\textsuperscript{2+} for phosphorylation of the enzyme and translocation to the intracellular membrane compartments; the Ca\textsuperscript{2+}-independent PLA2 (iPLA2) or group VI PLAs, does not require Ca\textsuperscript{2+} for activity and is also present in the cytosol\textsuperscript{15,16}, and the lysosomal PLA2 that functions in the lysosomes at low pH\textsuperscript{19}. The expression of PLA2 is regulated at the transcriptional level by mediators, such as cytokines and growth factors, including interferon-\(\gamma\), macrophage stimulating factor, tumor necrosis factor and epidermal growth factor\textsuperscript{17}. The PLA2 enzyme activity is 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)\textsuperscript{18}. In normal hearts, sPLA2 and cPLA2 are localized in the cytosol and cPLA2 and iPLA2 isozymes are localized in the cardiac SL membrane\textsuperscript{15,16,19}.

PLC Isozymes

The PLC isozymes play a central role in activating intracellular signal transduction pathways, especially during early key events in the regulation of various cell functions under normal and pathophysiological conditions\textsuperscript{20-27}. A number of different agonists, including norepinephrine (NE) and angiotensin II (ANG II), which are released by ischemic myocardium bind to their respective receptors on the cell surface resulting in G-protein (Gq subfamily) activation, which can lead to subsequent stimulation of PLC\textsuperscript{20-22}.

The activation of PLC results in the hydrolysis of phosphatidylinositol 4,5 bisphosphate (PIP\textsubscript{2}) to produce diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP\textsubscript{3}). While IP\textsubscript{3} may serve to enhance the SR Ca\textsuperscript{2+} release, DAG functions as a potent activator of most PKC isoforms, which in turn phosphorylate several cardiac proteins and stimulate Ca\textsuperscript{2+}-influx\textsuperscript{28-31}. Phenylephrine-induced transient increase in intracellular Ca\textsuperscript{2+}-concentration caused by Ca\textsuperscript{2+}-release from SR has been demonstrated to result in a transient suppression of L-type Ca\textsuperscript{2+}-current (I\textsubscript{Ca-L}) in rat ventricular cardiomyocytes\textsuperscript{32}.

Interestingly, this response is followed by a potentiation of I\textsubscript{Ca-L} that may involve PKC. In addition, this biphasic modulation of I\textsubscript{Ca-L} by phenylephrine can be blocked by prazosin, indicating that these responses are mediated by the GPCR, \(\alpha_1\)-adrenoceptor, and thus implicating a role for PLC.

The PLC family consists of 6 subfamilies: PLC \(\beta\), \(\gamma\), \(\delta\), \(\epsilon\), \(\zeta\) and \(\eta\)\textsuperscript{20-22,33-37} and are activated by different mechanisms\textsuperscript{38-44}. PLC \(\beta_1\), \(\delta_1\), \(\gamma_1\) and two forms of \(\epsilon\) are the predominant isoforms expressed in the heart\textsuperscript{43,45}. ANG II, \(\alpha_1\)-adrenergic agonists and endothelin-1 are stimulants of PLC \(\beta\) isozymes via the \(\alpha\) subunits of the heterotrimeric Gq subfamily; PLC \(\beta\) has also been shown to be activated by a G\(\beta\gamma\) dimer\textsuperscript{18}. A non-tyrosine kinase activation of PLC \(\gamma\) isoforms has been reported\textsuperscript{39} and the activation of PLC \(\gamma\) isoforms independent of tyrosine kinase has also been reported\textsuperscript{46}. The receptor initiated events for the activation of PLC \(\delta\) isoforms are considered to be mediated via transglutaminase II, G\(_{\alpha}\), a class of GTP binding protein\textsuperscript{47,48}. Although the PLC \(\delta\)-G\(_{\alpha}\) complex may be an important player in the signaling pathway that regulates calcium homeostasis and modulates physiological processes, PLC \(\epsilon\) isoforms are activated by Ras, Rho and Rap 2B, as well as by G\(_{\alpha_1}\)\textsuperscript{34,49}. The activation of PLC \(\zeta\) and \(\eta\) is far less characterized.

PLD Isozymes

The PLD isozymes hydrolyze phosphatidylcholine (PC) to produce phosphatidic acid (PA), which is considered to be an important lipid signaling molecule\textsuperscript{50,51}. PA can be dephosphorylated to DAG by the action of phosphatidate phosphohydrolase (PAP). Thus, both PLD and PAP can modulate the levels of PA and PLD-derived DAG in the heart. Different agents, such as NE, endothelin-1 and ANG II have been shown to increase the formation of PA in cardiomyocytes\textsuperscript{52,53}. The importance of PA in heart function is demonstrated from its ability to stimulate SL and SR Ca\textsuperscript{2+}-related transport systems\textsuperscript{54,55} and to increase the intracellular Ca\textsuperscript{2+} concentration in adult cardiomyocytes, as well as augment cardiac contractile activity in the normal heart\textsuperscript{56,57}. On the other hand, the \textit{in vivo} significance of the PLD-derived DAG remains to be defined\textsuperscript{56-58}.

Two mammalian PLD isozymes, PLD1 and PLD2, have been cloned exhibiting ~50% identity and have been shown to be differentially regulated\textsuperscript{59,60}. PLD1, which exhibits low basal activity, requires PIP\textsubscript{2} for its activity, and is activated by PKC and Rho small G-protein family members\textsuperscript{61-64}. PLD1 is localized to perinuclear regions such as endoplasmatic reticulum,
golgi apparatus, and endosomes. PLD2 is constitutively active and is the major SL PLD isozyme in the myocardium. PLD2 also requires PIP2 for its activity, but unlike PLD1, it is activated by unsaturated fatty acids such as arachidonic acid (AA) and oleate and is insensitive to the PLD1 activating factors. Figure 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. In addition, Fig. 2 depicts how remodeling of SL membrane and associated phospholipases could contribute to contractile defects in the diabetic heart; these events are described in the following sections.

Pathophysiology of diabetic cardiomyopathy

It is estimated that approximately 171 million people are affected by diabetes around the world and diabetes is predicted to affect 366 million people by 2030. Moreover, it is generally thought that about 30% of the population with diabetes remain undiagnosed in industrialized nations. Up to 10% of the diabetic population suffers from type 1 diabetes, whereas 85 to 90% of diabetics are affected by type 2 diabetes, making this disease the world’s most prevalent metabolic disease. The onset of type 1 diabetes occurs in children and type 2 diabetes occurs in adulthood; however, due to unbalanced diet and early obesity, type 1 diabetes (insulin-dependent) is also being identified in adults, whereas type 2 diabetes has been detected in children and teenagers.

Regardless of the type of diabetes, increased glucose in the plasma produces damage to small and large vasculature, which is considered to be the main factor for the increased mortality in diabetic populations. In fact, heart disease is the leading cause of death among diabetics. A disproportionately high prevalence of diabetes in African and Mexican Americans, when compared to Caucasians has been observed. In 2000, the top three ranked countries estimated to have the highest numbers of people with diabetes was India (31.7 million), China (20.8 million) and the USA (17.7 million). The top three countries are the same as those identified for 1995. It is notable that the same three countries have been predicted to remain in this notorious position with India (79.4 million), China (42.3) and the USA (30.3 million) in 2030. It should also be noted that the prevalence of diabetes is higher in men than women, but there are more women with diabetes than men. The urban population in developing countries is projected to double between 2000 and 2030.

Cardiovascular disease is responsible for 80% of deaths among diabetic patients much of which has been attributed to coronary artery disease. In fact, the incidence of heart disease is greater in the diabetic population than the non-diabetic population. However, the presence of a primary cardiomyopathy in diabetes has been long identified. It is estimated that 70 to 80% of diabetic patients die of cardiovascular complications, such as ischemic heart disease, atherosclerosis, hypertension, arrhythmias and congestive heart failure. In addition, diabetic cardiomyopathy has been shown to occur in the absence of coronary heart disease in diabetic
Diabetic cardiomyopathy is characterized by structural, cellular and molecular abnormalities, leading to diastolic dysfunction which progresses to left ventricular hypertrophy, followed by systolic dysfunction.

Figure 3 shows that diabetes is associated with the activation of neurohormonal systems, specifically how the activation of the renin angiotensin system (RAS) and sympathetic nervous system (SNS) contribute to diabetic cardiomyopathy in relation to alterations in the membrane function and signal transduction mechanisms. The activation of RAS, which results in the depletion of tissue antioxidant levels as well as impaired endothelial function, may also provide conditions favoring oxidative stress. In addition, activation of SNS and excessive production of catecholamines are also known to contribute to the occurrence of oxidative stress. Furthermore, oxidative stress induces subcellular remodeling that result in Ca²⁺-handling abnormalities and finally in contractile dysfunction in diabetic heart. The existence of diabetic cardiomyopathy is further attested by the high incidence of heart failure in diabetic patients seen in clinical trials, 26% in SOLVD (Studies Of Left Ventricular Dysfunction), 19% in ATLAS (Assessment Trial of Lisinopril And Survival) and 20% in V-HeFT II (Vasodilator-Heart Failure Trial II).

Phospholipid-mediated signaling in diabetic cardiomyopathy

The diabetes-induced changes in PL(A2) activities have been measured in several tissues, however, there is still a paucity of information regarding the status of PL(A2) isozymes in diabetic myocardium. Nevertheless, an increased membrane-associated iPL(A2) activity has been observed in the hearts of rats with streptozotocin (STZ)-induced diabetes, which may be due to the diabetes-induced increase in iPL(A2) mRNA level in rat myocardium. The increase in iPL(A2) activity is accompanied by an increase in LPC production. It is also shown that diabetes-induced changes in iPL(A2) activity and LPC production are reversed by insulin treatment of diabetic animals indicating that such changes in membrane phospholipid content and phospholipid hydrolysis may contribute to some of the alterations in myocardial function observed in diabetic patients.

Furthermore, the molecular species of major phospholipid classes in SL membrane of STZ-diabetic rats have also been examined. The content of plasmalogens is increased in all the phospholipid classes of diabetic SL membrane. PC and phosphatidylethanolamine are mostly enriched with molecular species containing linoleic acid and deprived of the molecular species containing AA. The molecular species of phosphatidyserine containing either AA or docosahexaenoic acid are less abundant in membranes from diabetic hearts than in membranes from controls. Insulin treatment of diabetic rats restores the species profile of phosphatidylethanolamine and overcorrects the changes in molecular species of PC. Accordingly, it is concluded that high SL level of plasmalogens and abnormal molecular species of glycerophospholipids may be critical for the membrane dysfunction and defective contractility of the diabetic heart.

Although ANG II and PKC have been implicated in cardiac dysfunction during diabetes, 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 α₁-adrenoceptor agonist, is ascribed to an increased PLC activity. We have earlier reported that total cardiac SL PLC activities are significantly decreased in STZ-induced chronic diabetic rats under in vitro assay conditions. In isolated cardiomyocytes, we have also observed a reduced concentration of basal as well as PA-induced IP₃ generation in diabetic rats, suggesting that decreased basal PLC activities in vivo may exist in diabetic cardiomyopathy. We have reported that a decrease in the total SL PLC in diabetes is associated

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**Fig. 3**—Activation of neurohormonal systems in diabetic cardiomyopathy that results in defects in phospholipases and occurrence of cardiac dysfunction [RAS, renin angiotensin system; SNS, sympathetic nervous system]
with a decrease in SL PLC \( \beta_3 \) activity, which immunofluorescence in frozen diabetic left ventricular tissue sections shown to be due to a decrease in PLC \( \beta_3 \) protein abundance; a 2-week insulin treatment of 6 wk diabetic animals partially normalizes these parameters\(^{96}\).

The significance of defective total PLC activities, including PLC \( \beta_3 \) activity and diminished levels of IP\(_3\) is that it may constitute a mechanism for the reported reduced force of contraction in response to \( \alpha_1 \)-adrenergic stimulation of the isolated papillary muscle\(^{97}\); however, an enhanced inotropic response to \( \alpha_1 \)-adrenergic stimulation in the isolated working heart from diabetic rats has also been reported\(^{98}\). On the other hand, a reduced production of PLC-derived DAG would affect several cellular processes\(^{99}\). While abnormalities in other signaling pathways occur during diabetes, in particular, the \( \beta \)-adrenoceptor induced increases in contractions and [Ca\(^{2+}\)]\(_i\) transients which are markedly diminished\(^{100,101}\), it can be suggested that an impairment of PLC signaling mechanisms may significantly contribute to a defective cardiac contractile performance during diabetes. Furthermore, depressed activities of other PLC isozymes in diabetic cardiomyopathy have also been observed\(^{102,103}\).

While the positive inotropic effect of PA on isolated perfused heart of STZ and alloxan-induced diabetic rats has been demonstrated\(^{104}\), 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 is correlated to an attenuated PA-induced IP\(_3\) generation in diabetic rat cardiomyocytes\(^{95}\). Insulin treatment of the diabetic animals results in a partial recovery of PA responses and it is 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\(^{105}\).

Several mechanisms can be proposed to explain the decrease in PLC activities in diabetic cardiomyopathy. In this regard, we have observed a reduction in the SL protein level of the major PLC isozyme, PLC\(\delta_1\) in diabetes (8-wks post-STZ injection), which is only partially corrected by insulin treatment (2-wks treatment of 6-wks diabetic animals) (Fig. 4A). Furthermore, the responsiveness of PLC\(\delta_1\) to PA is significantly depressed and is only partially corrected by insulin therapy (Fig. 4B). A decrease in SL PA formation due to an impaired PLD activity has also been reported\(^{106}\), which may be associated with a reduction in the stimulation of PLC.

Figure 5 shows that the reduced levels of SL membrane PA levels are not only due to low production by PLD activity, but also due to enhanced conversion of PA to DAG by phosphatidate phosphohydrolase activity; the net effect being a reduction in the PA content of the SL membrane. 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 defective PLD activity. Furthermore, oxidative stress, known to occur in diabetes\(^{74,80,82,83,96}\) may also contribute to a defective PLD activity\(^{107}\). An increase in myocardial DAG level has been reported in STZ-induced diabetic rats and in spontaneous autoimmune diabetic BB rats\(^{108,109}\).

**Fig. 4**—Activity and protein contents of the sarcolemmal associated phospholipase C \( \delta_1 \) isozyme in diabetes with or without insulin treatment [Values are means ± of 4 experiments. Assays were performed in duplicate as previously described\(^95\). *Significantly different (P<0.05) vs. control. #Significantly different (P<0.05) vs. control value + PA. PLC\(\delta_1\), phospholipase C \( \delta_1 \); IP\(_3\), total inositol phosphates; PA, phosphatidic acid]

**Fig. 5**—Sarcolemmal membrane levels of phosphatidic acid in diabetes [Values are means ± of 3 experiments. Assays were performed in duplicate as previously described\(^{106}\). *Significantly different (P<0.05) vs. control. PAP, phosphatidate phosphohydrolase; PLD, phospholipase D; PA, phosphatidic acid]
Increase in membrane DAG content has been shown to destabilize the membrane and structural transitions\textsuperscript{10,111} 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\textsuperscript{112}. Since SL PLC is inhibited by oxidants through reversible modification of the associated thiol groups\textsuperscript{113}, the depressed PLC activities seen in diabetes could, also in part be explained by the oxidant-induced alteration of thiol groups. \textit{In vitro} studies have demonstrated that LPC inhibits both SL PI 4 kinase and PI 4-P 5 kinase activities\textsuperscript{114}. This is highly significant as LPC accumulates in SL during diabetic cardiomyopathy, suggestive of a diminished synthesis of PIP\textsubscript{2} substrate for PLC. Furthermore, oxidants have also been shown to inhibit both SL PI 4 kinase and PI 4-P 5 kinase activities\textsuperscript{115}, as well as total SL PLC activity\textsuperscript{113}. However, while the total SL PLC activity in the diabetic heart is reduced\textsuperscript{94}, we have also reported that specific PLC isozymes could serve as therapeutic targets for cardioprotection under conditions of oxidative stress\textsuperscript{116}.

Figure 6 shows that the cardiac SL content of PIP\textsubscript{2} is reduced in diabetes due to depressed PI 4 kinase and PI 4-P 5 kinase activities that are not corrected by insulin treatment. 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\textsubscript{2} level may also contribute to the depressed cardiac contractility independent of the effects on PLC activities\textsuperscript{117}. It is pointed out that the hexosamine pathway is considered to inhibit phenylephrine-induced inotropy of the diabetic heart\textsuperscript{118}, 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.

Conclusions

From the evidence provided, it is clear that defects in the myocardial phospholipid-mediated signal transduction pathways occur in diabetes. However, contribution of these signaling systems relative to other myocardial signaling systems to the pathogenesis of cardiac dysfunction in diabetic cardiomyopathy remains to be established. Although some mechanisms responsible for the changes in the cardiac phospholipases have been presented they are still to be completely defined. The precise mechanisms for the regulation of cardiac phospholipase activities also remain to be completely understood. However, with the increasing complexity of phospholipid-mediated signal transduction mechanisms, the achievement of this outcome can be seen to be a major challenge. Not withstanding these limitations, it is reasonable to suggest that specific phospholipase isozymes might constitute additional therapeutic targets for drug discovery for the treatment of diabetic heart disease.

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