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REVIEW ARTICLE

Involvement of Phospholipase C in the Norepinephrine-Induced Hypertrophic Response in Cardiomyocytes

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Abstract

Norepinephrine (NE) is known to mediate cardiomyocyte hypertrophy through the G protein coupled α_1-adrenoceptor (α_1-AR) and the activation of the phosphoinositide-specific phospholipase C (PLC). Since the by-products of PLC activity are important downstream signal transducers for cardiac hypertrophy, the role of and the regulatory mechanisms involved in the activation of PLC isozymes in cardiac hypertrophy are highlighted in this review. The discussion is focused to underscore PLC in different experimental models of cardiac hypertrophy, as well as in isolated adult and neonatal cardiomyocytes treated with NE. Particular emphasis is laid concerning the α_1-AR-PLC-mediated hypertrophic signalling pathway. From the information provided, it is evident that the specific activation of PLC isozymes is a primary signalling event in the α_1-AR mediated response to NE as well as initiation and progression of cardiac hypertrophy. Furthermore, the possibility of PLC involvement in the perpetuation of cardiac hypertrophy is also described. It is suggested that specific PLC isozymes may serve as viable targets for the prevention of cardiac hypertrophy in patient population at-risk for the development of heart failure.

Key words: Phospholipase C isozymes; Norepinephrine; α_1-adrenoceptor; Cardiomyocytes; Experimental models of cardiac hypertrophy; Signal transduction.

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Introduction

Cardiovascular disease (CVD) remains the major cause of death worldwide and congestive heart failure (CHF) represents an enormous clinical, societal and economic burden.[1] In fact, according to the World Health Organization,[2] CVD is the leading cause of death globally, with an estimated 17.9 million deaths per year. Furthermore, > 75 % of CVD related deaths are due to heart attacks and strokes and about 30 % of these deaths occurring prematurely in people < 70 years of age. While it was estimated that there were over 37.7 million heart failure cases worldwide in 2016,[3] in 2020, the worldwide prevalence of heart failure was reported to be 64.34 million cases (8.52 per 1,000 inhabitants), accounting for 9.91 million years lost due to disability and 346.17 billion US $ expenditure.[4]

CHF is invariably associated with cardiac hypertrophy and changes in the shape and size of cardiomyocytes (cardiac remodelling) are considered to explain cardiac dysfunction in CHF. While the heart is known to adapt to increased work and haemodynamic load by increasing muscle mass as well as changing the size and shape of the heart, such a remodelling of the myocardium is compensatory at initial stages, but results in cardiac failure at late stages of the development.[4, 5] A moderate

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increase in the level of hypertrophic hormones including norepinephrine (NE) produces beneficial effects during early stages of cardiac hypertrophy, but prolonged exposure of the hearts to an excessive amount of NE produces deleterious actions at late stages of cardiac hypertrophy.[4, 5]

It is now well established that different subcellular organelles including the sarcolemma (SL) membrane, undergo varying degrees of changes in their biochemical composition and molecular structure during the development of cardiac hypertrophy as well as in the transition of cardiac hypertrophy to heart failure.[6-9] This SL remodelling occurs due to alterations in cardiac gene and protein expression as well as activation of different signalling proteins including phospholipases that are associated with the SL membrane.[10-12] The activation of phospholipase C (PLC) has a number of immediate consequences for signal transduction events in cardiomyocytes and thus has an integral role to play in SL and cardiac remodelling during the early stages of cardiac hypertrophy.[10-12] Although there are several hypertrophic agents that can activate PLC including angiotensin II, endothelin 1 and other growth factors, this brief review is intended to describe the involvement of specific PLC isozymes in the NE-induced hypertrophic response in cardiomyocytes and provide evidence that PLC isozymes may play an important role in the initiation of signal transduction processes involved in cardiac hypertrophy. In addition, evidence is provided to show that specific PLC isozymes may potentially be targeted for the prevention of cardiac hypertrophy and its ultimate transition to heart failure in patient population at-risk for the development of heart disease.

Phospholipase C isozymes and their regulation

The phosphoinositide-specific PLC enzyme is expressed in all mammalian cells and is critically involved in various signal transduction processes.[13, 14] Indeed, the activation of different PLC isozymes has been observed to be a key early event in the initiation of various cell functions.[13, 14] There are 13 families of PLC isozymes, which have been categorised into 6 classes. Earlier data on amino acid sequencing from cDNAs revealed the existence of PLC β, δ and γ isozymes,[15] but additional PLC isozymes, namely, ε, ζ, and η were discovered later.[16] Recently, this has expanded to 16 isozymes with the discovery of 3 atypical PLCs in the human genome.[14] It should be noted that there are four PLC β isozymes (β_1 to β_4), three PLC δ isozymes (δ_1, δ_3, δ_4), two PLC γ isozymes (γ_1, γ_2), one PLC ε isozyme, one PLC ζ, isozyme and two PLC η (η_1, η_2); these differ in their expression patterns in a variety of cells.[17] It is also pointed out that all PLC family members are a diverse group of isozymes that exhibit unique structures and cellular functions.[13] However, PLC is known to hydrolyse phosphatidylinositol-4,5-bisphosphate (PIP_2) to produce two second messenger molecules, namely inositiol-1,4,5-trisphosphate (IP_3) and *sn*-1,2-diacylglycerol (DAG).[18] IP_3 has been shown to trigger the release of Ca^{2+} from the intracellular stores, whereas DAG is known to activate protein kinase C (PKC) (Figure 1).

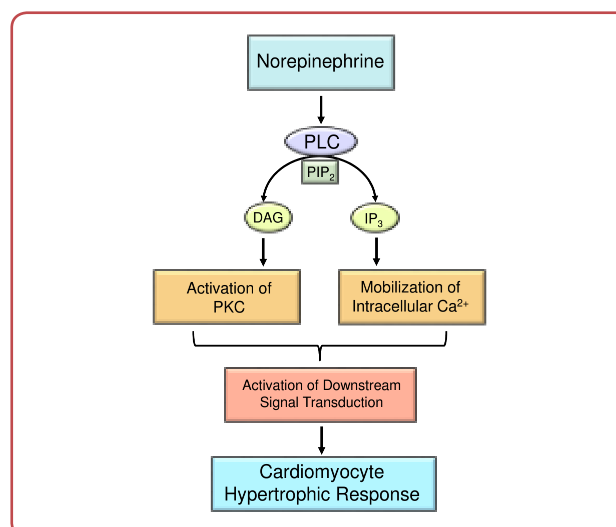

Figure 1: Involvement of phospholipase C signal transduction in the cardiomyocyte hypertrophic response to NE

PLC = phospholipase C; PIP_2 = phosphatidylinositiol-4,5-bisphosphate; DAG = sn-1,2-diacylglycerol; IP_3, inositiol-1,4,5-trisphosphate; PKC = protein kinase C

Indeed, the primary step of the signal transduction pathway for the activation of PKC involves the stimulation of PLC. It should be mentioned that specific PKC isozymes have also been implicated in the regulation of hypertrophic growth of cardiomyocytes.[19-22] Although the PLC family of isozymes signal through the same phospholipid hydrolytic products, each isozyme may contribute to distinct cellular functions.[13, 17] PLC isozymes are activated by a variety of factors including heterometric G proteins, small G proteins, receptor/non-receptor tyrosine kinases and calcium.[23] Among the PLC isozymes, PLC β and PLC γ are stimulated by receptor activation; PLC β by G-protein coupled receptors (GPCRs), including α_1-adrenoceptor (α_1-AR), whereas PLC γ by receptor tyrosine kinases.[24]

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PLC β, δ, γ and ε are expressed in adult ventricular cardiomyocytes.[25-30] PLC β family has four types of isozymes (β_1, β_2, β_3 and β_4).[22] While PLC β_1 and PLC β_3 isozymes have been extensively characterised in cardiac tissue, higher PLC β_4 mRNA expression levels as compared to PLC β_1, β_2, and β_3 have been reported in human left ventricular tissue.[31] α_1-AR agonists, including NE are relevant stimulators of PLC β isozymes via the α subunits of the heterotrimeric Gq subfamily;[32] PLC β has been shown to be activated by Gβγ dimer.[33] Interestingly, it was further demonstrated that, similar to the other three PLC β isozymes, PLC β_4 was activated by the α subunit of Gq, but not by the transducin α subunit. However, unlike other PLC β isozymes, PLC β_4 was not responsive to activation by Gβγ subunits.[33] It has recently been reported that the direct activation of PLC β by Gαq and/or Gβγ subunits mediates the signalling by Gq and some Gi couples GPCRs respectively, suggesting that the disruption of autoinhibitory interactions leads to increased PLC β activity.[34] It may also be noted that Gβγ has also been shown to directly interact and activate PLC ε.[35] The most abundant PLC isoform found in the heart, PLC γ_1 is cytosolic and is activated by growth factor receptor tyrosine kinases.[36] A non-tyrosine kinase mediated activation as well as GPCRs via non-receptor tyrosine kinase activation of PLC γ isozymes has also been reported.[37] Although PLC γ is activated through receptor tyrosine kinase, it seems that a reciprocal cross-talk between tyrosine kinase and Gqα may exist in cardiomyocytes,[38] linking α_1-AR with tyrosine kinase associated receptors.

PLC δ_1 is considered the predominant PLC isozyme associated to the SL membrane, because the N-terminal part of the pleckstrin homology domain of PLC δ_1 possesses a critical region rich in basic amino acid residues, which bind with high affinity to the polar head of PIP_2.[39] This property confers on the δ_1 isoenzyme a unique capacity of association with the plasma membrane, which is lost with single basic amino acid replacement by a neutral or acidic amino acid.[40] The α_1-AR initiated events for the activation of PLC δ isozymes are considered to be mediated by the dimeric G_h protein.[41-43] It should be mentioned that the PLC β_1 splice variant PLC β_{1b} and not he PLC β_{1a} associates with a Shank3 complex at the SL membrane via its splice-variant specific C-terminal tail,[44] and it appears that SL membrane localisation is central to the activation of PLC and downstream signalling events in response to the activation of GPCRs.[44]

PLC isozymes and the cardiomyocyte hypertrophic response

The role of PLC in the development of different types of cardiac hypertrophy has been documented; for example, the development of cardiac hypertrophy in stroke-prone spontaneously hypertensive rats has been suggested to involve an increase in the PLC signalling pathway.[45, 46] In addition, studies in neonatal rat cardiomyocytes stimulated with different hypertrophic stimuli, including NE, have shown an increased mRNA expression of PLC β isozymes.[47, 48] Stimulation of signalling pathways via Gqα provokes cardiac hypertrophy in cultured cardiomyocytes and transgenic mouse models overexpressing Gqα,[49-52] that may be linked to the activation of PLC. On the other hand, no correlation of hypertrophy to PLC activation in two other transgenic mouse lines expressing activated Gqα has been demonstrated.[53, 54] Recently, the activation of PLC β_3 mediated signal transduction has been reported in a rat model of cardiac hypertrophy induced by aortic constriction.[55] While the activation of PLC isozymes as an important signalling event in hypertrophy of the adult heart, a loss of PLC ε signalling in PLC ε knock out mice has been reported to sensitise the heart to development of hypertrophy in response to chronic isoproterenol treatment.[56] On the other hand, PLC ε depletion, using siRNA, reduces the hypertrophic response to NE as well as other hypertrophic stimuli in neonatal rat cardiomyocytes.[56] These authors also observed that PLC ε activity was required for hypertrophic development, yet PLC ε depletion did not reduce inositol phosphate production suggesting a requirement for localised PLC activity. It has also been suggested that a PLC ε - dependent component of β-adrenoceptor signalling in cardiomyocytes is responsible for the maintenance of contractile reserve and that loss of PLC ε signalling in PLC ε (-/-) mice sensitises the heart for the development of hypertrophy in response to cardiac stress.[57]

We have previously reported an increase in PLC isozyme gene and protein expression as well as activities in the hypertrophied rat heart subsequent to volume overload induced by arteriovenous shunt.[58, 59] It was demonstrated that specific increases in PLC β_1 and PLC γ_1 were associated with the hypertrophic stage in this model.[59] In contrast, PLC β_1 and Gαq protein levels have been reported to be unchanged during hypertrophy due to pressure overload induced by ligation of the de-

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scending thoracic aorta in the guinea pig.[60] However, translocation of PKC isozymes from cytosol to membranous fractions was elevated. These investigators suggested that PKC translocation occurred without changes in Gαq and PLC-β protein abundance and that it might be due to increases in Gαq and PLC β_1 activity rather than upregulation of expression,[57] but PLC β_1 activity was not determined in this study. It is pointed out that mechanical stress induced by cell stretching in neonatal cardiomyocytes has also been reported to increase PLC activity.[61] However, in this study no attempt was made to identify the PLC isozymes responsible for such responses. Since mechanical stretch is an initial factor for cardiac hypertrophy in response to haemodynamic overload (high blood pressure) and that increases in Gqα and PLC β_1 activities[61] as well as enhanced NE release from sympathetic nerves[62] are involved in pressure-overload hypertrophy, it is likely that α_1-AR activates PLC β isozymes under conditions of mechanical stress. It should also be noted that the involvement of α_1-AR-Gαq-protein-PLC-IP_3 signal transduction pathway in the development of NE-induced cardiac hypertrophy may be complimentary to other well-established mechanisms, namely β-AR-Gs protein-adenylyl cyclase-cyclic AMP for the induction of cardiac hypertrophy by catecholamines.[5, 9, 62]

It is interesting to note that the caveolae have a key role in signal transduction processes including an important role in the development of cardiac hypertrophy.[63, 64] In this regard, the α1-adrenoceptor, Gq, PLC β_1 and PLC β_3 have been found to be located exclusively to the same caveolin microdomain in the caveolar fraction isolated from rat heart.[65] It is pointed out that the NE-induced IP_3 generation in neonatal rat cardiomyocytes has been reported to be primarily due to α_1-AR-mediated activation of PLC β_1.[48] PLC β_1 exists as two splice variants, PLC β_{1a} and PLC β_{1b}, which differ only in their C-terminal sequences of 64 and 31 amino acids, respectively. While PLC β_{1a} is localised in the cytoplasm, PLC β_{1b} targets to the SL, which is enriched in caveolae, where α_1-AR signalling is also localised.[66] Furthermore, in cardiomyocytes, responses initiated by α_1-AR activation involve only PLC β_{1b}, thus the selective targeting of this splice variant to the SL membrane provides a potential target to reduce hypertrophy.[66] In this regard, the overexpression of PLC β_{1b} in neonatal cardiomyocytes was also shown to result in increases cell size and protein/DNA ratio as well as elevated atrial natriuretic factor (ANF) levels, indicating that the hypertrophic response due to the activation of the α_1-AR is mediated by PLC β_{1b} and thus may serve as a viable target for the limitation of cardiac hypertrophy.[67] Additionally, PLC β_4 gene expression levels have been reported to be increased in response to hypertrophic stimuli in mouse HL-1 cardiomyocytes thus indicating that PLC β_4 may also have a role to play in hypertrophic response in cardiomyocytes.[31]

It was reported earlier that NE increases in ANF gene expression and protein synthesis in adult rat cardiomyocytes, which are attenuated by a PLC inhibitor, U73122.[68] It was also observed that the NE-induced increase in ANF gene expression and protein synthesis were inhibited by prazosin, an α_1-AR blocker.[68] Furthermore, both prazosin and U73122 depressed the NE-induced increase in DAG production in cardiomyocytes. Taken together, it was determined that the α_1-AR mediated activation of PLC is involved in the hypertrophic response in cardiomyocytes. An extension to these observations[69] demonstrated that specific PLC isozymes may be involved in the cardiomyocyte hypertrophic response to NE. In this regard, while NE increased the activities as well as the mRNA levels of the predominant forms of PLC expressed in ventricular cardiomyocytes, β_1, β_3, δ_1 and γ_1, pre-treatment of adult rat cardiomyocytes with prazosin resulted in an attenuation of the NE-induced increases in PLC isozyme activities and gene expression (Figure 2).[69]

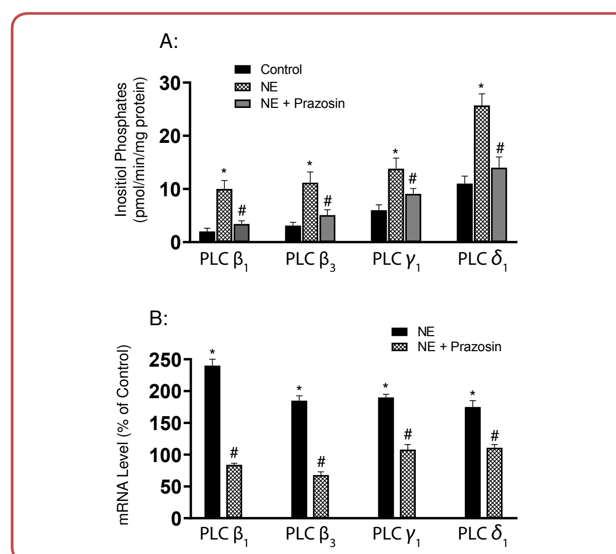

Figure 2: Phospholipase C (PLC) β_1, β_3, γ_1 and δ_1 activities and mRNA levels in cardiomyocytes treated with NE in the presence and absence prazosin

*Adult rat cardiomyocytes were treated with 5 μM NE for 2 h in the absence and presence of prazosin (2 μM). Values are mean ± SE of five experiments performed with five different cardiomyocyte preparations and expressed relative to GAPDH mRNA level. *Significantly different ($P < 0.05$) versus control; #significantly different ($P < 0.05$) versus NE. NE = norepinephrine; Data are based on the analysis of information in our paper[69]*

The involvement of these specific PLC isozymes in the cardiomyocyte hypertrophic response to NE was further substantiated by PLC gene silencing techniques using siRNA. Silencing of PLC β_1, β_3, δ_1 and γ_1, with siRNA resulted in the prevention of the NE-induced increase in ANF expression (Figure 3).[70]

In addition, cardiomyocyte protein synthesis, as evidenced by the incorporation of [^{3}H] phenylalanine, was markedly reduced in cardiomyocytes transfected with PLC isozyme siRNA[70] that was linked to a depression in the NE-induced increases in PLC isozyme activities (Table 1).[70]

The study of PLC and its involvement in cardiac hypertrophy under different pathophysiological conditions is both exciting and intriguing, but complex. It has for a long time been considered that the role of the α_1-AR in cardiac hypertrophy is a contributory factor, however, based on our studies as well as that of others, it seems that the activation of the α_1-AR-PLC signal transduction pathway may be a primary event in the initiation and pathogenesis of cardiac hypertrophy. It should be mentioned that the mechanisms of the regulation of PLC isozymes has also been examined. In this regard, it has been observed that the NE-induced increases in PLC isozyme gene expression occurs via a PKC- and ERK1/2- dependent signalling pathway and involves both *c-fos* and *c-jun* transcription factors.[69] Furthermore, since the PLC activity inhibitor, U73122, attenuated PLC gene expression, it was suggested that PLC isozyme activities may regulate their own gene expression. In addition, a reciprocal regulation of *c-fos* and *c-jun* and PLC isozyme gene expression may exist in cardiomyocytes.[70] Taken together, these observations suggest that PLC may be involved in the perpetuation of the hypertrophic response to NE. Indeed, the specific activation of PLC β, γ and δ isozymes, but loss of PLC ε, may be important elements in the initiation/increased sensitisation for cardiac hypertrophy as depicted in Figure 4.

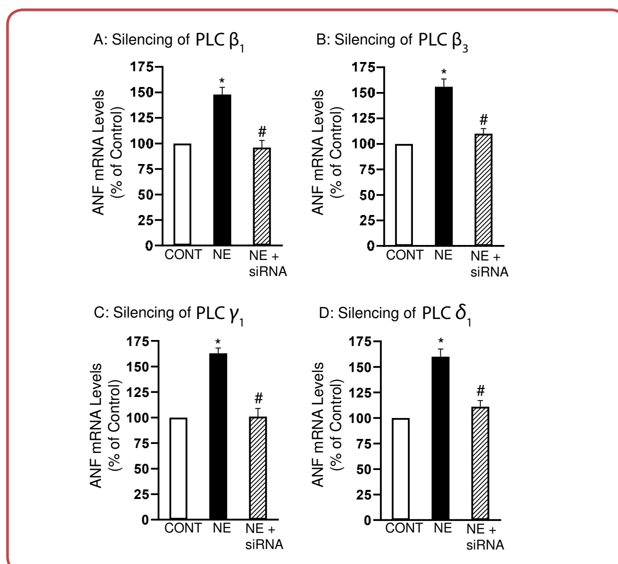

Figure 3: Inhibition of NE - induced increases in ANF mRNA levels in cardiomyocytes transfected with phospholipase C (PLC) isozyme siRNA

*Quantified data showing ANF mRNA levels relative to GAPDH mRNA level in cardiomyocytes transfected with 5 nM PLC isozyme (A) β_1, (B) β_3, (C) γ1 and (D) δ_1 siRNA and treated with NE (5 μM) for 2 hrs. Values are mean ± S.E. of five experiments performed with five different cardiomyocyte preparations. *Significantly different ($P < 0.05$) versus control; #significantly different ($P < 0.05$) versus NE. CONT = control; NE = norepinephrine; siRNA = small interfering RNA. Data are based on the analysis of information in our paper[70]*

Table 1: Inhibition of NE - induced increases in protein synthesis and phospholipase C (PLC) isozyme activities in cardiomyocytes transfected with PLC isozyme siRNA

A:	**Protein Synthesis ([^{3}H] phenylalanine incorporation, DPM)**
Control	9185 ± 820
NE	21046 ± 1132*
PLC β_1 siRNA	10171 ± 860#
PLC β_1 siRNA	11499 ± 778#
PLC γ_1 siRNA	10049 ± 752#
PLC δ_1 siRNA	9411 ± 764#

B:	**Inositol Phosphates (pmol/min/mg protein)**			
	PLC β_1	**PLC β_3**	**PLC γ_1**	**PLC δ_1**
Control	2.3 ± 0.5	2.5 ± 0.7	4.8 ± 0.7	8.1 ± 2.5
NE	10.7 ± 2.1*	13.3 ± 1.5*	15.1 ± 1.5*	22.0 ± 3.1*
siRNA	4.9 ± 1.7#	6.7 ± 2.0#	7.0 ± 2.3#	15.1 ± 2.0#

*Values are mean ± S.E. of five experiments performed with five different cardiomyocyte preparations. *Significantly different ($P < 0.05$) versus control; #significantly different ($P < 0.05$) versus NE. CONT = control; NE = norepinephrine; siRNA = small interfering RNA.*

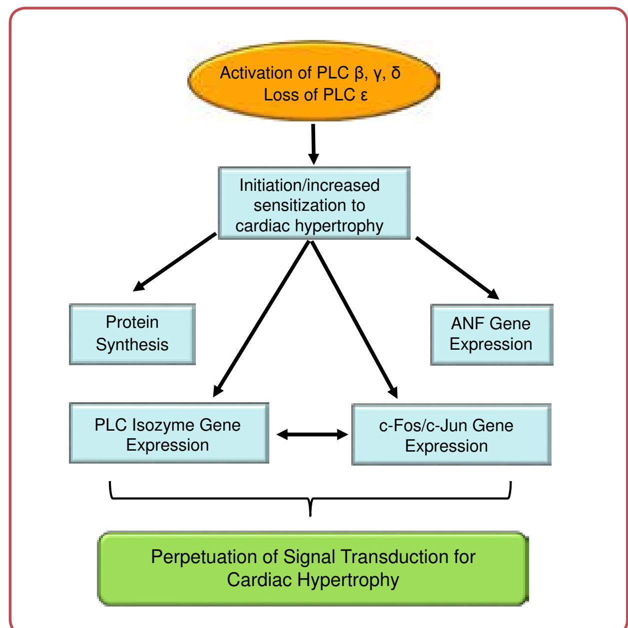

Figure 4: Sequence of events for the perpetuation of the hypertrophic response involving phospholipase C (PLC) isozymes

ANF = atrial natriuretic factor

Conclusion

This review has provided some evidence for the possible involvement of PLC in cardiac hypertrophy as well as identified some of the signal transduction mechanisms involved in the regulation of PLC isozyme gene expression and protein levels in the heart. Most of the available literature has predominantly described the role of PLC β isozymes in cardiac hypertrophy; however, as discussed, there are other PLC isozymes that are expressed in the heart, which may also have a distinct role to play in the cardiomyocyte hypertrophic response. Furthermore, the extent of any overlapping functionality of PLC isozymes, including the presence of PLC splice variants in cardiomyocytes needs to be explored. The role of PLC δ_1 and PLC γ_1 as well as their activation in cardiac hypertrophy also require further investigation, particularly since the cardiac specific overexpression of G_h results in a unique hypertrophy phenotype that is independent of GPCR- induced activation of PLC.[71] While some studies have shown prazosin in mitigating the progression of cardiac hypertrophy to heart failure[72-77] a selective modulation of PLC (isozyme gene expression, protein contents and activities) and regression of cardiac hypertrophy remains to be established in different types of animal models of cardiac hypertrophy. It is pointed out that losartan, an angiotensin II type 1 receptor blocker, can selectively attenuate the increase in PLC isozyme gene expression (PLC β_1, β_3 and δ_1) during the development of cardiac hypertrophy subsequent to arteriovenous shunt.[59] These changes were associated with a regression of cardiac hypertrophy as evidenced by a reduction in the left ventricle/body weight ratio.[59] It should be mentioned that PLC isozyme activities in this study was not determined, which are the key element of PLC signalling function and thus some caution should be exercised in the interpretation of these findings. However, the regression of cardiac hypertrophy by pharmacological agents can be seen to be associated with the selective inhibition of some PLC isozymes.

While the aforementioned discussion has pertained to the role of PKC as a downstream effector of the PLC-derived DAG in the cardiomyocyte hypertrophic response to NE, it should be mentioned that the other by-product of PLC hydrolytic activity, IP_3, has also been implicated as a key component of the cell signal in cardiac hypertrophy.[78, 79] An interesting role of the Golgi in cardiac hypertrophy has recently emerged[80, 81] suggesting that the PLC-derived phosphoinositide and DAG production is required for the activation of protein kinase D during cardiac hypertrophy and that PIP_2 is not the preferred substrate, unlike the plasma membrane phosphatidylinositol 4-phosphate. Overall, it can be suggested that specific PLC isozymes may be involved in the initiation of signal transduction processes for the development of cardiac hypertrophy and thus might constitute additional therapeutic targets for drug discovery for the treatment of cardiac hypertrophy and its progression to heart failure in at-risk patients.

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Conflict of interest

None.

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