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1. INTRODUCTION

1.1. General introduction and cardiac hypertrophy:

Cardiovascular diseases (CVD) leading to heart failure which in turn causes death all over the world. It accounts for 16.7 million deaths annually which are estimated to be almost 1/3rd of all deaths worldwide (Agrawal et al., 2010; Barry et al., 2008; Banerjee and Cruickshank, 2006). Among different CVD, left ventricular hypertrophy (LVH) or simply hypertrophy is regarded as an independent risk factor for heart failure development and considered to be one of the main causes of heart failure worldwide (Agrawal et al., 2010; Anamourlis et al., 2006). Hypertrophy can be defined as an increase in heart mass. The increase in heart mass is largely due to an increase in ventricular weight (Bernardo et al., 2010).

The heart is composed of cardiac myocytes, non-myocytes (e.g. fibroblasts, endothelial cells, mast cells, vascular smooth muscle cells) and surrounding extracellular matrix (ECM) (Zak, 1984). Cardiac myocytes are specialized muscle cells composed of bundles of myofibrils. The myofibrils have repeating micro-anatomical units called sarcomeres (contractile unit of the heart) (Gregorio and Antin, 2000). In mammals, at birth or soon after, it is generally believed that most cardiac myocytes lose their ability to proliferate and growth occurs primarily as a result of an increase in myocyte size (Soonpaa et al., 1996). Even though ventricular cardiac myocytes make up only 1/3rd of the total cell number, they account for 70–80% of the heart’s mass (Zak, 1984). In the adult, the growth of the heart is usually closely matched to its functional load. In response to changes in functional load, the heart triggers a hypertrophic response to counterbalance the increase in wall stress (Zak, 1984). Different kinds of hypertrophy develop as a result of physiologic, genetic and environmental factors (Evangelista et al., 2003). Different humoral factors like various growth factors, vasoactive peptides, cytokines and catecholamines contribute to develop cardiac hypertrophy while mechanical stress is thought as the primary trigger for the development of cardiac hypertrophy in the overloaded heart (Peng et al., 2006). Hypertrophy may also be developed as a result of an increase in cardiac workload due to hypertension, valvular disorders and ischemic events in the myocardium (Harada et al., 2007). Although at the beginning cardiac hypertrophy is an essential adaptive response of the heart or myocardium to various stresses (mechanical, metabolic or genetic) to preserve cardiac function but in the long run it ultimately threatens the affected patient with progression to
heart failure and sudden death (Frey and Olson, 2003; Takeda et al., 2010; Izumiya et al., 2006). Prolonged external load evolves a state of decompensated hypertrophy which results in loss of contractility and cardiac dilation (Izumiya et al., 2006).

1.2. Types of cardiac hypertrophy:

Cardiac hypertrophy can be classified in three main types:

a. Developmental hypertrophy:

During embryogenesis, growth of the heart occurs primarily through proliferation of cardiac myocyte and non-myocyte cells of the heart. However, soon after birth, cardiac myocytes withdraw irreversibly from the cell cycle and subsequent growth of the heart occurs predominantly through hypertrophy rather than myocyte hyperplasia.

b. Physiological Hypertrophy:

This type of hypertrophy is reversible and normal which enhances cardiac performance to meet the increased metabolic demands of our body during postnatal development, chronic exercise and pregnancy (Agrawal et al., 2010). Increase in heart size is not associated with any kind of cardiac damage and tissue homogeneity of the myocardium is maintained in this kind of hypertrophy i.e. proportionality of vascular, muscular and interstitial compartments are maintained (Weber and Brilla, 1991; Barry et al., 2008). Increase in ventricular wall thickness together with matched increase in ventricular chamber dimensions characterizes this kind of cardiac hypertrophy.

Physiological hypertrophy can be sub classified as concentric or eccentric based on changes in shape that are dependent on the initiating stimulus (Pluim et al., 2000; Grossam et al., 1975). Isotonic exercise (e.g. running, walking, cycling and swimming) involves movement of large muscle groups. The profound vasodilatation of the skeletal muscle vasculature that is involved produces eccentric hypertrophy by increasing venous return to the heart and volume overload (Pluim et al., 2000; Zak, 1984). This hypertrophy is characterized by chamber enlargement and a proportional change in wall thickness. In contrast, isometric or static exercise (e.g. weight lifting) involves developing muscular tension against resistance with little movement. Reflex and mechanical changes cause a pressure load on the heart rather than volume load resulting in concentric hypertrophy (Pluim et al., 2000; Zak, 1984) (Figure 1).
c. Pathological Hypertrophy:

Pathological hypertrophy occurs in response to various pathological stresses or signals like, inflammation, neurohumoral activation, arterial stenosis and cardiac injury (Agrawal et al., 2010). Although, initially it seems to be an adaptive response of the heart to normalize wall stress and to improve cardiac performance, prolonged exposure to these stimuli leads to decompensated and compromised cardiac performance and heart failure. This hypertrophy is irreversible and involves several structural changes in the myocardium which leads to decline in left ventricular performance and eventual heart failure. Unlike physiological hypertrophy, myocardial tissue homogeneity is lost greatly in pathological hypertrophy (Weber and Brilla, 1991). Increased workload is associated with increase in ventricular wall thickness along with a decrease in ventricular chamber diameter which reduces ventricular chamber volume and thereby decreased ventricular function. The major stimuli for this type of hypertrophy constitutes hypertension, myocardial infarction (MI), genetic polymorphism, altered cardiac metabolism and also diabetes (Barry et al., 2008).

Pathological hypertrophy also can be sub divided as concentric or eccentric growth. A pathological stimulus causing pressure overload (e.g. hypertension, aortic stenosis) produces an increase in systolic wall stress that results in concentric hypertrophy (hearts with thick walls and relatively small cavities) (Grossam et al., 1975). In contrast, a pathological stimulus causing volume overload (e.g. aortic regurgitation, arteriovenous fistulas) produces an increase in diastolic wall stress and results in eccentric hypertrophy (hearts with large dilated cavities and relatively thin walls) (Pluim et al., 2000; Grossam et al., 1975) (Figure 1).
1.3. **Distinct feature of physiological and pathological cardiac hypertrophy:**

Despite comparable increase in heart size, physiological and pathological hypertrophy is associated with distinct structural and functional, metabolic and biochemical and molecular features.

Under physiological hypertrophy condition the collagen networks provides structural support to the adjoining myocytes which enhance cardiac pump function. Pathological hypertrophy is associated with cell death (apoptosis, necrosis) and loss of cardiomyocyte is replaced with (known as fibrosis). The main collagen present in cardiac tissue during fibrosis is type 1 collagen. Excessive accumulation of collagen stiffens the ventricle which impairs contraction and relaxation and reduces cardiac pump function (Gunasinghe and Spinale, 2001).

In the normal healthy heart, fatty acid oxidation is the main metabolic pathway that is responsible for 60-70% adenosine triphosphate (ATP) generation; while glucose and lactate metabolism pathway is responsible for 30% of the ATP production. The normal heart is
capable of switching energy substrates depending on workload and concentration of substrate in the bloodstream. During an adaptive condition (e.g. fasting, exercise etc) which allows the heart to produce a continuous supply of ATP (van der Vusse et al., 1992). Thus physiological hypertrophy induced by exercise training is characterized by enhanced fatty acid and glucose oxidation (Gertz et al., 1988). Pathological cardiac hypertrophy is always associated with decrease in fatty acid oxidation and increase in glucose metabolism (Allard et al., 1994; Christe and Rodgers, 1994; Davila-Roman et al., 2002). During foetal cardiac development, oxygen supply is limited and fatty acid transport and metabolism are impaired due to carnitine deficiency and delayed maturation of enzymes involved in fatty acid oxidation. Thus glucose is the primary substrate used by the foetal heart to generate ATP (Ostadal et al., 1999).

It was recognized that physiological hypertrophy (induced by exercise training/thyroid hormone) was associated with elevation in myosin ATPase activity and enhancement of contractility, whereas pathological hypertrophy (induced by renal hypertension, aortic banding) was associated with decreased myosin ATPase activity and depressed contractile function (Wikman-Coffelt et al., 1979; Rupp, 1981). The investigators reported a distinct pattern of gene expression in the two hypertrophy models (Iemitsu et al., 2001). Molecular changes during physiological hypertrophy include increase in expression of α- myosin heavy chain (α-MHC) and cardiac α-actinin genes. In contrast, alteration in gene expression pattern during pathological hypertrophy involves activation of different foetal genes; atrial natriuretic factor (ANF), β-myosin heavy chain (β-MHC), skeletal alpha actin (SKA) and also activation of several immediately early genes like, c-jun, c-fos and c-myc which are considered as markers of cardiac hypertrophy (Agrawal et al., 2010).

Human and animal studies have demonstrated that certain factors are preferentially released in response to pathological and physiological stimuli. It is well recognized that insulin growth factor-1 (IGF-1) is released during postnatal development and in response to exercise training (Yeh et al., 1994; Conlon and Raff 1999; Koziris et al., 1999; Serneri et al., 2001b; Perrino et al., 2006), and IGF-1 level in the heart was increased in swim-trained rats (Scheinowitz et al., 2003). Furthermore, production of cardiac IGF-1 (but not Ang II or ET-1) was increased in professional athletes compared with control subjects (Serneri et al., 2001b). In contrast, pressure overload is associated with elevated levels of Ang II, catecholamines and ET-1 (Arai et al., 1995; Yamazaki et al., 1999; Rapacciuolo et al., 2001; Yayama et al.,
2004) and cardiac formation of Ang II was increased in heart failure patients with hypertrophied hearts (Serneri et al., 2001a).

1.4. Commonly used experimental models of physiological and pathological cardiac hypertrophy:

Studies utilizing genetic models alone or in combination with morphologically distinct models of hypertrophy have become very powerful tools for understanding molecular pathways responsible for different forms of heart growth in vivo. Commonly used experimental models of pathological hypertrophy include pressure overload (constriction/banding of the renal, abdominal, ascending or transverse aorta), volume overload (aortocaval shunt) and mini pump infusions of vasoactive substances (e.g. isoproterenol, angiotensin II). Physiological models include treadmill exercise, freewheel running and chronic swim training (Bernardo et al., 2010). Thus, generation of animal models of physiological and pathological hypertrophy allowed investigators to delineate signalling proteins that appear to play distinct roles in regulating physiological and pathological cardiac hypertrophy. Signalling pathways responsible for the induction of physiological and pathological cardiac hypertrophy are more complex and are not identical.

1.5. Signalling pathways in the development of physiological and pathological cardiac hypertrophy:

Though, physiological and pathological cardiac hypertrophy has distinct characteristics, it is unclear whether these two forms of hypertrophy were induced by distinct biochemical pathways. Transgenic and knockout technology in combination with surgical and exercise models has proven very powerful in delineating signalling cascades that play a role in regulating physiological and pathological growth.

To date, the best characterized signaling cascades responsible for mediating physiological and pathological cardiac hypertrophy are the IGF-1- phosphoinositide 3-kinase [PI3K, (p110α)]-Akt pathway and Gαq signaling (downstream of G protein-coupled receptors (GPCR) activated by Ang II, ET-1 and catecholamines) respectively (Figure 2). Other signaling pathways associated with physiological cardiac hypertrophy and/or protection include the gp130/JAK/STAT pathway, thyroid hormone signaling, and heat shock transcription factor-1 (HSF1). In contrast, pathological hypertrophy has also been associated
with abnormalities leading to enhanced PI3K (p110γ), mitogen activated protein kinases (MAPKs), protein kinase C (PKC), protein kinase D (PKD) and calcineurin (Bernardo et al., 2010).

**Figure 2:** A schematic overview of pathological and physiological hypertrophy outlining key differences in initiating stimuli, signaling pathways, cellular responses and cardiac function.

1.6. **Protein Kinase C (PKC):**

1.6.1. **Characteristics and classification:**

The term "protein kinase C" usually refers to the entire family of isoforms (Churchill et al., 2008; Steinberg, 2008). Protein kinase C (PKC) is a multifunctional, cyclic nucleotide-independent protein kinase that phosphorylates serine and threonine residues in many target proteins. This enzyme was identified in bovine cerebellum by Nishizuka and co-workers (Takai et al., 1977) as a protein kinase that phosphorylated histone and protamine. Since its discovery, much interest has been shown in PKC and its role in signal transduction. PKC is known to comprise a large family of enzymes that differ in structure, cofactor requirements
and function. PKC enzymes are activated by signals such as increases in the concentration of diacylglycerol (DAG) or calcium ions (Ca^{2+}).

The structure of PKC isozymes includes conserved regions (C1-C4) that are interrupted by variable regions (V1-V5). The general structure of a PKC molecule consists of a catalytic and a regulatory domain found at the C- and N-terminus respectively. Catalytic domain consisting of C3 region (required for ATP binding) and C4 region (required for substrate-binding and catalysis). The catalytic domain can be active in the absence of cofactors after proteolytic removal of the regulatory domain by cleavage in the V3 region. The regulatory domain, which is responsible for dependence on cofactors, contains an auto-inhibitory pseudo-substrate region as well as sequences that mediate the interactions of PKC with phospholipids, DAG/phorbol esters, and specific anchoring proteins. The Ca^{2+} dependency is mediated by the C2 region while phorbol ester binding requires the presence of two cysteine-rich zinc-finger regions within the C1 domain.

There are at least 12 PKC isoforms that are classified into three subfamilies according to the structure of the N-terminal regulatory domain which determines their sensitivity to the second messengers Ca^{2+} and DAG. Subfamilies are conventional/classical (c), novel (n) and atypical (a) (Figure 3).

a. **Conventional PKC** isoforms are α, βI, βII, and γ. They require Ca^{2+}, DAG and a phospholipid such as phosphatidylserine (PS) for activation. This isoform contain a C1 domain, a tandem repeat that binds either DAG or phorbol ester and a C2 domain that binds the headgroup of PS in a Ca^{2+} dependent manner. The presence of these domains in conventional PKC isoforms explains their requirement for DAG and Ca^{2+} for their activation.

b. **Novel PKC** isoforms are δ, ε, η and θ and require DAG but do not require Ca^{2+} for activation. Thus, conventional and novel PKC isoforms are activated through the same signal transduction pathway as phospholipase C. This isoform contain a functional C1 domain and a non-functional C2 domain that does not contain the residues required for Ca^{2+} binding.

c. **Atypical PKC** isoforms are ζ and τ / λ, require neither Ca^{2+} nor DAG for activation. This isoform does not contain functional C1 and C2 domains and are thus DAG independent and Ca^{2+} independent.
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1.6.2. Activation:

PKC isoforms remain associated with the plasma membrane, in a dormant state, until covalently modified at distinct sites of the PKC protein. A critical event in the regulation of PKC activity is the phosphorylation of three highly conserved positions within the catalytic domain (Dutil et al., 1998; Dempsey et al., 2000). The first rate limiting phosphorylation occurs at the activation loop of the enzyme (Dutil et al., 1998; Dempsey et al., 2000). The upstream kinase responsible for the initial phosphorylation of PKC isoforms is 3-phosphoinositide-dependent protein kinase-1 (PDK)-1 (Dutil et al., 1998). PDK-1 phosphorylates the activation loop of conventional, novel, and atypical PKC isoforms (Nakanishi et al., 1993; Dutil et al., 1998; Chou et al., 1998). Following PDK-1-dependent phosphorylation of the activation loop, auto-phosphorylation is facilitated at two key COOH-terminus sites of the protein; a turn motif conserved among all PKC isoforms (Thr500 Thr641) and a hydrophobic phosphorylation motif (Ser660 PKCβII) conserved in conventional and novel PKC isoforms (Dutil et al., 1998). The atypical PKC isoforms differ

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**Figure 3**: Schematic diagram of primary structures of PKC family members showing domain composition and activators. The PKC family of isoforms consists of three classes: the classical (α, βI βII, and γ), novel (δ, ε, η and 0) and atypical (ζ and ι / λ).
by the insertion of an acidic Glu residue in place of Ser660. Posttranslational modification of PKC isoforms at the above sites is a prerequisite for activation by second messengers. Phosphorylation at the activation loop alters the conformation of the kinase, facilitating auto-phosphorylation at the COOH terminus (Dutil et al., 1998). However, phosphorylation at the activation loop of conventional PKC isoforms does not regulate maximal kinase activity (Dutil et al., 1998). The later property is dependent upon the interaction of regulatory domains with DAG and PS, resulting in the release of the auto-inhibitory pseudo-substrate domain from the active site (Dutil et al., 1998). Conversely, phosphorylation of the activation loop of the atypical PKC isoforms serves as a molecular switch to turn on catalytic activity (Edwards and Newton, 1997). Although phosphorylation dependent activation may occur in the absence of cofactors, phospholipid mixtures containing (3, 4, 5-IP3), promote this reaction (Dutil et al., 1998).

Mature PKC isoforms are released from the membrane into the cytosol and maintained in an inactive state by binding an auto-inhibitory sequence, known as pseudo-substrate. Membrane recruitment in response to DAG, PS or phorbol ester provides the energy for release of the pseudo-substrate from the substrate binding site, activating PKC for downstream signaling (Dempsey et al., 2000).

The activity of PKC is controlled by its compartmentalization within the cell. Mochly-Rosen (1995) indicated that specific anchoring proteins localize the kinases to their sites of action. These include 'receptors for activated C-kinase' (RACKS), annexins and other cytoskeletal proteins. For a protein to be considered a RACK, certain criteria must be fulfilled: (i) it should bind PKC in the presence of activators; (ii) PKC binding to recombinant RACK1 should not be inhibited by a pseudo-substrate peptide; (iii) binding of PKC to RACK1 should be saturable and specific; and (iv) RACKS should contain a sequence homologous to a PKC binding motif (Webb et al., 2000).

1.6.3. Distribution and localization:

The expression and distribution of PKC isoforms varies markedly between cells and tissues (Nishizuka, 1988). PKC-α is ubiquitously expressed whereas others seem to be restricted to certain tissues. PKC-δ was present in brain, heart, spleen, lung, liver, ovary, pancreas, and adrenal tissues. PKC-ε was present in brain, kidney, and pancreas. PKC-ζ was
present in most tissues, particularly the lung, brain, and liver. Both PKC-δ and PKC-ζ showed some heterogeneity of size among the different tissues. PKC-α was present in all organs and tissues examined. PKC-βI and -βII were present in greatest amount in brain and spleen.

Substrate accessibility and function may be regulated by isoform localization to different subcellular compartments. The biological functions of PKC have mostly been linked with events occurring at the plasma membrane level and/or in the cytoplasm, because PKC isoforms are thought to be associated with the cytoskeleton in an inactive state and, after maturation (phosphorylation), they translocate to the plasma membrane (or the membrane of cytoplasmic organelles) to become fully activated in the presence of specific cofactors (Hug and Sarre, 1993). Co-expression and parallel activation of multiple PKC isoforms, isoform interdependence and cross-talk and overlapping isoform effects are potential confounders for measuring PKC signaling. However, increasing evidence has implied a role for PKC in nuclear functions, suggesting this may be a pathway to communicate signals generated at the plasma membrane to the nucleus (Rosenberger et al., 1995). Almost every PKC isoform has been reported to be present in the nucleus in some cellular system under certain conditions, either as the consequence of translocation from cytoplasm or as a resident enzyme (Martelli et al., 2003). Classical PKCs and novel PKCs translocate from the cytosol to membranous sites upon activation with DAG. TPA and certain other phorbol esters mimic DAG in that they can bind and activate all PKCs, except the atypical PKCs (Konig et al., 1985).

1.7. PKC and cardiac diseases:

Protein kinase C are involved in a number of signal transduction mechanisms by phosphorylating specific proteins essential for modifying different biological processes (Nishizuka, 1984). In the heart, these signal transducing proteins are engaged in the regulation of cardiac function, cation transport, myocardial metabolism, gene expression, cellular growth and cell apoptosis (Wang et al., 1999; Feuerstein and Young, 2000). Their mode of activation involves the transfer of the γ-phosphate group from ATP to hydroxyl groups of serine/threonine residues of protein serine/threonine kinases or tyrosine residues of protein tyrosine kinases (Sadoshima et al., 1995). Protein kinases act directly by invoking a biological response immediately after activation or by initiating branching or linear cascades of signal amplification and integration causing an eventual biological response (Sugden, 1995). In the heart, the majority of PKC appears in α and β forms (Nishizuka, 1986;
Bogoyevitch et al., 1993). PKC has significant involvement in a variety of signaling pathways mediating heart function so it is important to consider the type of cardiac dysfunction or disease. PKC has been shown to have increased myocardial activity associated with cardiac dysfunction in the diabetic heart (Liu et al., 1999).

Based on in vivo and in vitro experiments using phorbol esters as general PKC agonists, PKCs have long been implicated in cell proliferation, survival, and programmed death (Murray et al., 1997). In cultured cardiomyocytes, PKCs regulate contractility and hypertrophy (Dorn et al., 2000). However, there are at least 12 different isoforms of PKC, according to molecular cloning studies and the multiplicity of family members produces varied cellular responses depending upon isoform activity and physiological context. In cardiac tissues, PKC isoform expression differs with species, cell type, and developmental stage, with most adult mammalian myocardia expressing PKC-α, PKC-β1, PKC-βII, PKC-δ, PKC-ε, and PKC-λ/ζ (Sabri and Steinberg, 2003). The activity of any given PKC isoform is dependent upon its expression level, its localization within the cell and its phosphorylation state (Malhotra et al., 2001). Each of these factors is regulated in cardiac disease. Despite these complexities and the differences between experimental models and human syndromes, studies of myocardial hypertrophy or heart failure largely report similar overall findings: PKC-α and PKC-β are upregulated, PKC-ε is either upregulated or preferentially activated, and levels of PKC-δ and PKC-λ/ζ do not change (Bowling et al., 1999). However, this correlative approach does not distinguish primary pathological effects from secondary compensatory events and simultaneous regulation of multiple PKC isoforms with different subcellular destinations, substrates and cellular effects precluded assignment of individual pathological consequences based on associations alone.
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1.8. Background of the research work:

Myocardial hypertrophy is a major cause of mortality and morbidity throughout the world. Physiological (due to exercise, swimming) or pathological (due to hypertension, obesity or valve disorder, ischemic events) load to the myocardium leads to cardiac hypertrophy. Different types of hypertrophy develop as a combined effect of various humoral, mechanical, biochemical, genetic as well as environmental factors (Evangelista *et al.*, 2003; Peng *et al.*, 2006; Harada *et al.*, 2007). A number of pathophysiological changes occur during progression of cardiac hypertrophy. These changes include apoptosis, necrosis and change in extracellular matrix, myocyte alignment and contractile dysfunction (Anversa *et al.*, 1996; Colucci, 1997; Bishop *et al.*, 1998; Mittmann *et al.*, 1998). All these parameters, singly or in unison, result in compromised cardiac function that eventually leads to heart failure.
Recent research into the role of PKC in cardiac tissue has indicated that it has an important role in stimulating hypertrophy. This was demonstrated by the ability of agonist-mediated hypertrophy to be stopped only as a result of the inhibition of PKC in an experiment in situ. However, in further in vivo research using mice, the transgenic overexpression of any one type of PKC isoform showed no effect on cardiac growth and the inhibition of that isoform showed no effect on hypertrophic response to increased cardiac pressure. Research has shown that removing PKC isoform improved the hearts ability to contract (Dorn and Force, 2005).

In summary, research is pointing in the direction that PKC’s role in cardiac tissue has more impact as a regulator of hypertrophy. However, there has not been any significant attempt to compare the PKC isoform in two etiologically different forms of cardiac hypertrophy so far. So, till date there are no possible explanations regarding activation of PKC in two etiologically different forms of cardiac hypertrophy forms.