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Cardiac Hypertrophy and Heart Failure Development Through Gq and CaM Kinase II Signaling

Shikha Mishra, B.S.[1], **Haiyun Ling, PhD**[2], **Michael Grimm, MD PhD**[2], **Tong Zhang, MD PhD**[2], **Don M. Bers, PhD**[2], and **Joan Heller Brown, PhD**[2,*]

[1]Department of Biomedical Sciences, UCSD

[2]Department of Pharmacology, UCSD

Abstract

The molecular events associated with the development of pathological hypertrophy have been shown to be stimulated through G-protein coupled receptors (GPCRs) that activate Gq signaling pathways in neonatal cardiomyocytes and in transgenic (TG) and knockout (KO) mice. We demonstrated that CaMKII, a multifunctional Ca^{2+} regulated protein kinase, was activated through GPCR and $InsP_3$ mediated Ca^{2+} release and suggested that CaMKII was a downstream mediator of Gq -coupled hypertrophic signaling. This was supported by the demonstration of CaMKII activation by pressure overload (TAC) and induction of hypertrophy by transgenic (TG) CaMKII expression. CaMKII also phosphorylates Ca^{2+} handling proteins including the ryanodine receptor (RyR2), phosphorylation of which markedly increases sarcoplasmic reticulum (SR) Ca^{2+} leak. Increased RyR2 phosphorylation is associated with heart failure development in CaMKII TG mice, and mice genetically deleted for CaMKII (KO) have attenuated RyR2 phosphorylation, SR Ca^{2+} leak and heart failure development following long term TAC. Genetic ablation of CaMKII also decreases development of heart failure in Gq TG mice, and decreases infarct size, while improving functional recovery in mice subject to ischemia/reperfusion and preventing adverse remodeling following coronary artery occlusion. The underlying mechanisms are currently under study.

Keywords

CaMKII; Hypertrophy; Heart Failure; GPCR; ischemia/reperfusion

Involvement of CaMKII in cardiac hypertrophy

Cardiac hypertrophy is a mechanism by which the heart responds to extrinsic signals or injury in an effort to minimize cardiac wall stress. Physiologic hypertrophy is exemplified by the heart's adaptive response to athletic conditioning, while pathologic hypertrophy occurs in response to disease states such as hypertension or myocardial infarction (1). Pathologic cardiac hypertrophy is characterized by increased cell size, changes in protein synthesis, cardiac remodeling, myofilament reorganization and increased expression of fetal

[*]**Correspondence:** Joan Heller Brown, Department of Pharmacology, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0636 USA, Fax 858-822-4011, Phone 858-822 5858, jhbrown@ucsd.edu .

genes (2). In the neonatal rat ventricular myocyte (NRVM) model of hypertrophy, GPCR agonists such as norepinephrine (NE), endothelin-1 (ET-1), phenylephrine (PE) or prostaglandin F2 alpha (PGF2α) mimic these responses, inducing increases in cell size, reorganization of sarcomeric proteins and re-expression of fetal genes such as ANF, BNP, β-MHC and α skeletal actin (3-10). Signaling through these GPCRs is initiated by coupling to the heterotrimeric G protein Gq (11;12), and expression of the α subunit of Gq (Gαq) in NRVMs in the absence of agonist can also induce hypertrophy as evidenced by marked increases in cell size, hypertrophic gene expression and myofilament organization (13-15). In addition, hypertrophy can be elicited *in vivo* by cardiac transgenic Gαq overexpression (16). There is also evidence that Gq activation mediates hypertrophy *in vivo* in response to pressure overload (TAC). This was first shown in mice with cardiac specific overexpression of a specific Gαq inhibitor peptide, in which there was a reduced hypertrophic response to transverse aortic constriction (TAC) compared to control animals (17). In addition, mice with cardiac-specific deletion of Gαq family proteins show no ventricular hypertrophy in response to pressure overload (18).

The downstream target of Gαq is phospholipase C (11;12) and early work from our laboratory demonstrated that hypertrophic agonists induce phosphoinositide (PI) hydrolysis in NRVMs (7;19-21). PI hydrolysis results in the generation of diacylglycerol and activation of protein kinase C (PKC) as well as formation of inositol trisphosphate ($InsP_3$) which releases Ca^{2+} . Numerous laboratories including our own have examined and suggested involvement of PKC as well as various MAP kinase cascades in hypertrophic signaling (1;22;23). We also suggested that Ca^{2+}/CaM dependent protein kinase II (CaMKII) serves as a downstream mediator of GPCR induced hypertrophy based on the finding that inhibition of CaMKII with KN-93 blocked phenylephrine-induced hypertrophy in NRVMs (24). CaMKII is activated in response to elevated Ca^{2+} and its interaction with Ca^{2+}/ Calmodulin. How GPCR agonist induced PI hydrolysis would activate CaMKII in the heart is not clear. One possibility is via effects of PKC on Ca^{2+} channels, resulting in enhanced Ca^{2+} entry and release. Another could be via $InsP_3$ mediated Ca^{2+} release. In either case, these GPCR mediated changes in Ca^{2+} release would be superimposed upon the large and highly controlled changes in intracellular Ca^{2+} cycling that occur as part of excitation-contraction coupling. We recently addressed the question of whether localized, rather than global increases in Ca^{2+} induced by the Gq receptor coupled hypertrophic agonist ET-1 might lead to activation of CaMKII and elicit hypertrophic responses. This possibility was suggested by the observation that in cardiomyocytes, type 2 $InsP_3$ ($InsP_3R2$) receptors are concentrated in the nucleus (25) and that a splice variant of the predominant cardiac CaMKII isoform, CaMKII δ_B , contains a nuclear localization sequence which targets it to the nucleus (26;27). We determined in this study that CaMKII can be activated through an $InsP_3$ receptor dependent mechanism by demonstrating that either ET-1 or the $InsP_3$ receptor agonist adenophostin increased CaMKII autophosphorylation and that this response was attenuated by inhibition of $InsP_3$ receptor signaling using 2-APB (28). We also used myocytes from $InsP_3R2$ knock out mice (29) to show that CaMKII was not activated by ET-1 in the absence of $InsP_3R2$ (28). We further demonstrated that CaMKII activation by localized $InsP_3$ dependent increases in Ca^{2+} led to HDAC export from the nucleus whereas a more global increase in Ca^{2+} did not (26). Class II HDACs including HDAC regulate activity of the transcription factor MEF2 thereby affecting hypertrophic gene expression (30;31). Thus the concept of excitation-transcription coupling, whereby agonist-induced increases in nuclear Ca^{2+} activate CaMKII to mediate HDAC dependent gene transcription, was proposed (Figure 1).

Role of CaMKII in *in vivo* hypertrophy

To examine the possibility that CaMKII might be a mediator of cardiac hypertrophy development following pressure overload, CaMKII activation was measured in mice subject to TAC. We observed increases in autophosphorylated CaMKII (indicative of kinase activation) at 1 to 7 days post TAC (32-34). To determine whether increased CaMKII activity was a sufficient stimulus to induce hypertrophy, we generated cardiac specific CaMKII δ transgenic (TG) mice using the α-myosin heavy chain (αMHC) promoter. Both CaMKII δ_B and δ_C TG mice were generated. The CaMKII δ_B splice variant differs from δ_C by the inclusion of a nuclear localization sequence (26). Immunohistochemical analysis indicated that CaMKII δ_B was largely limited to the nucleus, while CaMKII δ_C was primarily cytosolic (27;33;35). There was cardiac enlargement at eight weeks in both CaMKII δ_B and δ_C TG mice compared to WT mice and both mice showed increases in expression of hypertrophic genes such as ANF, BNP, β-MHC and α skeletal actin (33;35;36). Thus both nuclear and cytosolic CaMKII δ overexpression induce hypertrophic responses.

Our earlier *in vitro* studies suggested that CaMKII, activated by localized nuclear $InsP_3$ signaling, phosphorylated HDAC within the nucleus, promoting its export to the cytosol to enhance gene expression (36-39). The *in vivo* data suggested, however, that CaMKII δ can also phosphorylate HDAC when expressed in the cytosol, preventing it from returning to the nucleus and repressing gene expression (36). Consistent with this hypothesis, we saw increases in cytosolic HDAC and transcriptional activation of MEF2 dependent gene expression in both CaMKII δ_B and δ_C TG mice (36, Figure 1).

Role of CaMKII in heart failure

While both CaMKII δ_B and CaMKII δ_C TG mice exhibited hypertrophy which ultimately became maladaptive, the CaMKII δ_C mice showed a remarkably rapid progression to heart failure, with all of its commonly associated phenotypic changes (27;33;35). CaMKII δ_C TG mice showed premature death compared to wild type mice with only 50% survival at 15 weeks of age, and displayed progressive cardiac enlargement and ventricular dilation at 6-13 weeks. This was coupled with a marked loss of ventricular function measured by fractional shortening (33;40). In addition, specific changes in Ca^{2+} handling proteins were seen in the CaMKII δ_C TG mice that were not recapitulated in the CaMKII δ_B line (33;36;40). One notable finding in the CaMKII δ_C TG mice was increased association of CaMKII with the ryanodine receptor RyR2 (33;40). There was also increased phosphorylation of RyR2 at Ser2815, a known CaMKII phosphorylation site (33;40). Ca^{2+} sparks in cardiomyocytes isolated from CaMKII δ_C TG mice were significantly increased in number, frequency and width, leading to a 4.3 fold increase in diastolic SR “calcium leak” compared to wild type cardiomyocytes. Indeed, the “leak” was so substantial that SR Ca^{2+} content, measured by the caffeine-induced Ca^{2+} transient, was decreased by more than 60% in the CaMKII δ_C TG mice relative to wild type (40).

To determine whether the decreased SR Ca^{2+} content that resulted from the SR Ca^{2+} leak was a major contributor to contractile dysfunction and heart failure development in the CaMKII δ_C TG mice, these mice were crossed with phospholamban (PLN) knockout mice generated by the Kranias lab (41). PLN deletion restored SR Ca^{2+} loading and Ca^{2+} transients in the CaMKII δ_C TG as anticipated. Remarkably however, PLN KO/TG mice did not show improvement, but rather an exaggerated heart failure phenotype compared to the δ_C TG mice i.e. they showed greater mortality, ventricular dilation, and myocyte death (42). Characterization of cardiomyocytes from the PLN KO/TG mice revealed that recovery of the SR Ca^{2+} load (facilitated by PLN deletion) coupled with the increased P-RyR (due to

CaMKII δ_C overexpression) conspired to markedly increase Ca^{2+} sparks. Of significant interest, this increase in diastolic SR Ca^{2+} leak was shown to result in mitochondrial Ca^{2+} loading, which did not occur when the SR Ca^{2+} leak was minimized (42). Experiments using ryanodine to minimize the leak and Ru360 to reduce mitochondrial Ca^{2+} overload showed that survival of cardiomyocytes from the PLN KO/TG mice was markedly enhanced (42). Current studies are aimed at determining the importance of the mitochondrial PT pore and other mechanisms by which heart failure progression occurs in the CaMKII δ_C TG and double mutant mice.

Is CaMKII required for hypertrophy and transition to heart failure?

To further explore the role of CaMKII δ in hypertrophy and heart failure we generated CaMKII δ knockout mice (43). The CaMKII δ knockout mice (KO) exhibited no gross baseline changes in ventricular structure and function. When cardiac hypertrophy was induced by 2-week isoproterenol stimulation, comparable hypertrophy developed in WT and KO mice (unpublished observation). In addition, CaMKII δ deletion had no effect on hypertrophy induced by 2-week transverse aortic constriction (TAC), as evidenced by gravimetric analysis, echocardiographic parameters and cell size measurement (43). Consistent with this we found equivalent upregulation of hypertrophic gene expression in WT and KO mice subject to TAC, and there was no difference in the phosphorylation of the CaMKII target, HDAC5. The observation that CaMKII δ was not required for the development of hypertrophy in response to pressure overload was unexpected and led us to examine involvement of other kinase signaling pathways that could compensate for CaMKII δ deletion. The gamma (γ) isoform of CaMKII is normally present at low amounts in the heart, and its expression is not affected by CaMKII δ deletion (43). However, confirming a previous report (32), we found that expression of CaMKII □ was increased by pressure overload and this occurred to a similar extent in WT and KO mice (43). We are currently testing the hypothesis that maintenance of hypertrophy in the CaMKII δ KO mice is explained by the concomitant upregulation in expression and activation of CaMKII γ. We also find that protein kinase D (PKD) activation is increased by pressure overload and to similar extents in WT and KO mice (43). Thus, an alternative hypothesis is that PKD, which has been implicated in the development of TAC induced hypertrophy (44), and also functions as an efficacious kinase for class II HDACs including HDAC5 (38;39), provides signals that mediate cardiac hypertrophy in the absence of CaMKII δ.

Remarkably, while hypertrophy is intact in the CaMKII δ KO mice, the transition from hypertrophy to heart failure is not (43). Specifically, WT mice 6 weeks post TAC show a range of phenotypic changes (e.g. chamber dilation, ventricular dysfunction, lung edema, cardiac fibrosis, apoptosis) associated with heart failure development. All of these responses are significantly attenuated in CaMKII δ KO mice, and survival after TAC is dramatically improved (43). These salutary effects of CaMKII δ deletion on the response to long-term pressure overload are observed in the absence of any diminution in the initial development of hypertrophy, as indicated above. Thus we suggest that CaMKII δ deletion does not prevent decompensation simply because it prevents the development of pathological hypertrophy, but rather that it plays a more fundamental role in the transition to heart failure.

Role of CaMKII in Ca^{2+} handling and heart failure development

It is well accepted that disturbed Ca^{2+} handling contributes to the development of heart failure. Heart failure is associated with significant quantitative changes in the level of expression of intracellular Ca^{2+} regulatory proteins including the sarcoplasmic or endoplasmic reticulum calcium ATPase2 (SERCA2), the predominant cardiac IP_3 receptor (IP_3R2) and the ryanodine receptor (RyR2) (40;45). Expected changes in expression of these

proteins were observed in WT mice subject to long term TAC but were attenuated in CaMKII δ KO mice. Of particular interest, WT mice showed increases in IP_3R2 and decreases in RyR2 expression, modest at 2 weeks and more significant at 6 weeks after TAC (43). The increase in IP_3R2 and decrease in RyR2 was attenuated in CaMKII δ KO mice. Thus the elevated IP_3R2 to RyR2 ratio associated with TAC induced heart failure is markedly diminished in the KO mice (43). Taken together, these results suggest that CaMKII δ contributes to the altered expression of Ca^{2+} regulatory proteins during the development of pressure overload-induced heart failure.

Of further interest, we demonstrated that the fraction of RyR2 phosphorylated at the CaMKII site was increased in response to TAC both prior to and during development of heart failure but that this did not occur in CaMKII δ KO mice (43). We suggest that increases in CaMKII-phosphorylated RyR2 contribute to the decompensation of pressure overload induced heart failure in WT mice. As mentioned earlier, RyR2 phosphorylation in CaMKII δ_C TG mice enhanced diastolic Ca^{2+} leak and was suggested to be causally related to the development of heart failure (40). To assess SR Ca^{2+} leak as a functional correlate of the RyR2 phosphorylation induced by TAC, we measured Ca^{2+} sparks in cardiomyocytes isolated from WT and KO mice. There was no difference in the Ca^{2+} spark frequency in WT versus KO mice at baseline. A highly significant difference in spark frequency was observed, however, in mice subjected to 6-week TAC, with SR Ca^{2+} leak increasing in cardiomyocytes from WT but not KO mice (43). We suggest that the ability of CaMKII δ deletion to prevent TAC-induced SR Ca^{2+} leak underlies its salutary effect in this mouse model of pressure overload induced heart failure.

In the knockout model described above, CaMKII activity is absent beginning in early development and deleted in tissues other than the heart. We have used cardiac specific CaMKII δ KO mice to reproduce our observation that heart failure development is blunted, thus the effect we see is due to loss of CaMKII in cardiomyocytes (unpublished). Whether inhibiting CaMKII after initiation of hypertrophy can prevent heart failure development or improve its course is a critical question that is currently under study. We are also attempting to determine if CaMKII mediated SR Ca^{2+} leak can be blocked pharmacologically and if this has beneficial effects on heart failure development. Of further interest is the possibility that the β-adrenergic receptor signals through CaMKII, at least under more chronic conditions, and that this may contribute to the efficacy of β-adrenergic blockade in heart failure. The evidence for a role of CaMKII in β-adrenergic induced heart failure was recently reviewed (46). Extrapolating from mouse models to the therapy of human heart failure is clearly premature, but we are encouraged by the phenotypic similarities between heart failure induced through and dependent upon CaMKII δ and that seen clinically and in other genetic heart failure models.

Role of CaMKII in Gq induced hypertrophy and heart failure

Our previous studies demonstrated that Gαq expression in NRVMs stimulates cardiac hypertrophy (13-15), consistent with the hypertrophic effect of Gq-coupled receptor agonists (3-10;47). Pioneering experiments from Dorn's laboratory established that this occurs *in vivo*, i.e. overexpression of Gαq induces a hypertrophic phenotype similar to that elicited by pressure overload (16;48). While Gαq expression *in vivo* induces hypertrophy, high levels of Gαq expression or accentuated stress signaling elicited by aortic banding or pregnancy in Gαq TG mice is associated with hypertrophic decompensation (14;16;48). Progression of Gαq-mediated hypertrophy results in part from induction of cardiac apoptosis. Gαq TG mice that develop peripartum cardiomyopathy were shown to exhibit massively increased apoptosis (14;49). A mitochondrial death pathway involving the Bcl2 family protein Nix, was implicated in Gq induced apoptosis (13-15;50;51). We have also shown that enhanced

Gαq signaling induced by expression of a constitutively activated form of Gαq causes cardiomyocyte apoptosis (13-15). Recent observations suggest that CaMKII expression is increased in Gαq TG mice and that CaMKII is activated by Gαq expression in NRVMs, consistent with the possibility that CaMKII signals downstream of Gq activation (manuscript in preparation, Ling et al). Accordingly we asked whether genetic deletion of CaMKII protected against development of heart failure induced by Gαq overexpression. Gαq TG mice were crossed with CaMKII δ KO mice. Echocardiography demonstrated that CaMKII δ deletion attenuated the left ventricular chamber dilation and dysfunction seen in Gq mice. Hemodynamic measurements also confirmed that there was improved heart function in the double mutant mice compared to the Gαq TG mice heart functions. Other hallmarks of heart failure such as arrhythmias, cardiac fibrosis and apoptosis were also significantly prevented by CaMKII δ deletion in mice overexpressing Gαq (manuscript in preparation, Ling et al).

CaMKII in I/R injury and in response to MI

Reperfusion is essential to salvage ischemic tissue, but it also contributes to myocardial damage referred to as reperfusion injury. CaMKII inhibition with KN-93 or through cardiac expression of the CaMKII inhibitor AC3-I has been shown to protect against ischemic damage resulting from myocardial infarction (MI) or *ex vivo* ischemia/reperfusion (I/R) injury (52;53). Using our recently generated CaMKII δ KO mice, the role of CaMKII in ischemic injury was examined further. WT and KO mice were subjected to *ex vivo* global I/R (22min /1hour), left anterior descending coronary artery (LAD) occlusion to induce ischemia for 1-hr followed by reperfusion for 24 hours (*in vivo* I/R), or permanent LAD occlusion for 6 weeks (MI). CaMKII δ deletion significantly reduced infarct size following either *ex vivo* or *in vivo* I/R and there was improved recovery of cardiac function in the absence of CaMKII. In response to MI, CaMKII δ ablation was found to inhibit the development of left ventricular dilation as assessed by echocardiography. Preliminary results indicate that adverse remodeling was diminished and cardiac function improved in KO compared to WT mice (manuscript in preparation, Ling et al). These findings provide further evidence that CaMKII δ plays a deleterious role in the development of myocardial injury and heart failure in MI and I/R as well as pressure overload models.

Is there a differential role for CaMKII δ_B and δ_C in heart failure?

In an effort to better understand the mechanism by which CaMKII δ_C TG mice developed heart failure, we crossed the CaMKII δ_C mice with several other genetic mouse models that had potential to rescue the heart failure phenotype. As described above, replenishing the SR Ca^{2+} load via PLN ablation actually exacerbated the phenotype (42). Interestingly two other rescue strategies, inhibition of CaMKII δ_C at the SR with SR targeted AIP (54) and preventing mitochondrial permeability transition pore formation by cyclophilin D ablation (55) also failed to rescue the heart failure seen in CaMKII δ_C TG mice (Huke S et al. in revision; Elrod J et al. submitted). Increased dilation or apoptosis also appear to accompany the increased lethality in these models. These results are consistent with the suggestion that CaMKII δ_C is involved in pro-apoptotic signaling and sensitizes towards activation of mitochondrial death pathways (56). Several recent studies have also demonstrated that whereas CaMKII δ_C is proapoptotic, CaMKII δ_B has protective effects (57;58). In this regard it is notable that CaMKII δ_B TG mice do not rapidly transition from hypertrophy to heart failure, although secondary changes in protein phosphatase activity ultimately affect SR Ca^{2+} uptake and predispose to ventricular dilation (35). Future studies using CaMKII δ_B versus δ_C subtype specific KO and TG mice will allow us to gain insight into the function of the individual CaMKII δ splice variants and their contribution to Ca^{2+} handling, transcriptional regulation and cell viability, and how this impacts the development of heart

failure. It will also be informative to examine changes in CaMKII expression and activity in human heart failure patients in light of the potential for opposing effects of the CaMKII δ_B and δ_C subtypes, as well as for compensatory effects of increased CaMKII □

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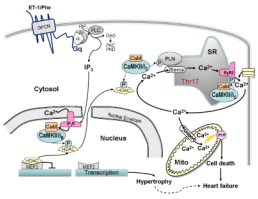

Figure 1.

Activation and targets of CaMKII δ in hypertrophy and heart failure. Abbreviations: **ET-1**, Endothelin-1; **Phe**, Phenylephrine; **GPCR**, G-Protein Coupled Receptor; **α**, α subunit of the Gq-protein; **β□**, β□ subunit of the Gq-protein; **PIP_2**, Phosphatidylinositol 4,5-Bisphosphate; **PLC**, Phospholipase C; **DAG**, Diacylglycerol; **PKC**, Protein Kinase C; **PKD**, Protein Kinase D; **CaM**, Calmodulin; **PLN**, Phospholamban; **SR**, Sarcoplasmic Reticulum; **SERCA**, SR Ca^{2+} ATPase; **CaMKII δ**, Calcium Calmodulin Dependent Protein Kinase II; **RyR2**, Ryanodine Receptor 2; **IP_3**, Inositol (1,4,5)-Trisphosphate; **HDAC**, Histone Deacetylase; **IP_3R**, IP_3 Receptor; **Mito;** Mitochondria; **PTP**, Permeability Transition Pore; **MEF2**, Myocyte Enhancer Factor-2.