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Cardiovascular diseases remain the main cause of death in the western world. Many of these diseases such as coronary artery disease, hypertension, and diabetes eventually lead to chronic heart failure (HF). HF occurs when the heart cannot pump adequate blood to meet metabolic demands. The primary initial response of cardiac myocytes to increased work is a balance between hypertrophy and apoptosis, or programmed cell death.[1,2] Apoptosis is thought to augment the progression to HF from compensatory hypertrophy simply by decreasing the number of functional myocytes present in the heart. Discovering what processes or pathways contribute to this tipping of the scale from compensatory hypertrophy to HF remains a mystery and is paramount to helping treat HF patients effectively.

It has been well documented that hypertrophy can be induced by endogenous and exogenous agents that act on G protein-coupled receptors (GPCRs) that specifically activate the heterotrimeric G protein Gq.[1] Early pioneering studies showed that α_1-adrenergic receptor (α_1-AR) stimulation elicited hypertrophy in response to norepinephrine (NE) in neonatal rat ventricular myocytes (NRVMs).[3] Subsequently, many other Gq-coupled receptor agonists have been shown to cause hypertrophy including angiotensin II (AngII), endothelin-1 (ET-1), thrombin and prostaglandins. This hypertrophy is considered to be compensatory at first, aiding the heart in maintaining cardiac output and supplying needed oxygenated blood to distant organs. However, this compensatory hypertrophy can become deleterious and the heart begins its slide into failure. At what point the scale tips in favor of maladaptation and HF versus adaptive cardiac hypertrophy is the research goal of many investigators and discovering molecules or pathways that can act on both sides of this scale is essential in understanding the transition to HF.

In this issue of the *Journal of Molecular and Cellular Cardiology* Howes et al.[4] attempt to separate cell survival from hypertrophy in response to direct Gαq stimulation. The authors demonstrate the ability of the α-subunit of Gq to activate two separate downstream pathways independently: hypertrophy and anti-apoptotic mechanisms. The current manuscript suggests that direct Gαq stimulation is a candidate that may play a role in mediating a balance between these two pathways with the primary mechanistic target the activation of Akt (also known as protein kinase B).

Numerous studies to date have demonstrated that GPCRs are important in activating the phosphoinositide-3 kinase (PI3K)/Akt pathway, which is a cellular pro-survival signaling axis. PI3K phosphorylates and activates Akt which subsequently phosphorylates glycogen synthase-3β (GSK3β) inhibiting its apoptotic effects on NF-AT, β-catenin and

caspase-3 activation.[5] Upon activation of a given GPCR, its α subunit and βγ subunits dissociate and each component can activate a number of downstream molecules and therefore, both could theoretically be involved in Akt activation. In vitro studies have shown that both Gαq and Gβγ (from Gi-coupled receptors) can activate Akt.[6] Recent in vivo experiments have also shown the importance of Gβγ activating the PI3K–Akt pathway in the hypertrophying heart as transgenic mice expressing a Gβγ sequestering peptide (βARKct) had significantly less activation of PI3K in myocardium after pressure-overload compared to non-transgenic control mice indicating that PI3K activation, upstream of Akt activation, is βγ-subunit dependent.[7] The current manuscript is important as it clearly illustrates that Gαq alone can activate Akt in a PI3K-dependent manner. Future studies will need to be completed in order to verify that different combinations βγ subunits, which may not be sequestered by the βARKct, are not involved.

In addition to their more classically appreciated signaling cascades, it is now appreciated that GPCRs, including those that are Gq coupled such as AngII AT1 receptors, can also activate PI3K via transactivation of receptor tyrosine kinases (RTKs) such as the receptor for epidermal growth factor (EGFR).[8] The pathophysiological significance of this novel Akt signaling is not known but could be important especially in conditions of cardiac stress. Moreover, it is not completely understood whether Gαq alone can do this. Another level of complexity is added when one considers that a given GPCR is capable of coupling and activating multiple G proteins. Therefore, it has been difficult to definitely and directly ascribe the role of individual G proteins in PI3K-mediated Akt activation or whether this is RTK dependent. In this current study, overexpressed Gαq alone is able to activate the tyrosine kinase, Src, which phosphorylates and activates the EFGR and causes PI3K-dependent Akt phosphorylation.[4] The transactivation of the EGFR by Gαq is similar in mechanisms to the GPCR–RTK cross-talk that occurs in cardiac fibroblasts where activation of β_2-ARs causes cell proliferation in an Src and EGFR-dependent manner.[9] The end result of Gαq-Akt activation in Howes et al. [4] [4] is an increase in cardiomyocyte survival when these cells are exposed to the pro-apoptotic agent, 2-deoxyglucose (2DOG).

The premise that Gαq can have cellular pro-survival features is intriguing considering that initial studies ascribed Gαq overexpression as a condition causing the heart to fail in vivo and also inducing apoptosis after cardiac stress.[10, 11] These studies in transgenic mice also demonstrated a dose-dependent on Gαq expression in cardiac hypertrophy and pathology. Lower levels had minimal effects on cardiac growth while higher levels either caused significance hypertrophy and HF or even death due to ventricular failure.[10] This finding led to further studies comparing wild-type Gαq overexpression and overexpression of a constitutively active mutated form of Gαq (GqQ209L) in cardiac myocytes.[11] Data showed that although both wild-type Gαq and GqQ209L caused hypertrophy of cells, only the constitutively active GqQ209L produced apoptotic cell death. Importantly, those studies and the current study within this issue may in fact be complementary. Previously, it was shown that the constitutively active mutant GqQ209L decreased levels of phosphatidylinositol bisphosphate (PIP_2), a substrate for PI3K, thus decreasing the ability of PI3K to activate Akt that occurs downstream.[4] This depletion is explained by the increased phospholipase C (PLC) activity caused by GqQ209L

expressing cells. Therefore, it was concluded that decreased levels of PIP_2 due to enhanced Gq activity in heart failure limit the availability of PIP_2 for PI3K/Akt signaling.[4] The current manuscript by this group provides novel mechanistic insight into how Gαq overexpression activates Akt and provides anti-apoptotic effects, presumably in conditions where PIP_2 is not limiting.

Howes et al. provides evidence that not only can Gαq mediate cardiac hypertrophy through PKC and MAPK signaling, it can also directly signal via the cardioprotective PI3K pathway. This manuscript is also one of the first to describe that Gq can transactive the EGFR in a PKC- and Ca^{2+}-independent manner, which is interesting since the primary downstream actions of Gq-coupled GPCRs are tied to activation of phospholipase C, PKC and Ca^{2+} mobilization.[6] It will be interesting to investigate the mechanisms underlying this novel signaling pathway of Gαq and whether this is due to specific spatial and temporal activation of Gq-coupled receptors in the myocyte.

Another important finding from this study is the ability to separate the hypertrophic pathways stimulated by more classical Gq that are EGFR independent (MAPK and calciuneurin) from the cardiomyocyte protective pathways that are dependent upon novel Gαq–EGFR activation.[4] At which point activation of Gαq prefers one pathway versus the other raises an interesting question. In the early stages of cardiovascular disease it could be hypothesized that there may be equal signaling down the respective hypertrophic and the anti-apoptotic pathways in myocytes resulting in cardiac hypertrophy with adequate function, as would occur with compensatory hypertrophy. In the chronic setting, as circulating levels of AngII, ET-1, and NE increase and exposure is prolonged, activation of Gq-coupled receptors increases, and the "stealing" of PIP2 by the hypertrophic pathways may become prevalent resulting in a decrease in the ability of Gαq to activate the Src/EGFR dependent PI3K/Akt signaling cascade. With a subsequent decrease in phosphorylated (i.e. activated) Akt (phospho-Akt) and its cardioprotective benefits, cell survival would be compromised with a decrease in myocytes the ventricles will become dysfunctional.

The dissection of these pathways provides valuable information on the mechanisms that are associated with Gαq stimulation. However, it is important to keep in mind that although Gq signaling is increased through particular GPCRs in hypertrophy and HF, the expression levels of Gαq are not increased, unlike what has been shown for other G proteins including Gαi.[12] In the current study, the authors wanted to investigate Gq signaling specifically therefore used the overexpression strategy as they did not want to complicate the results by adding a Gq-coupled agonist. As discussed above this is important because many Gq-coupled receptors can couple to other G proteins such as Gi or also can directly activate other proteins including ion channels and thus, the authors chose to study "pure" Gαq signaling. However, it is important that effects from these confounding variables be considered within the context of Gq-coupled signaling in vivo in the failing heart as you cannot have simple Gαq activation without activation of a GPCR and thus in HF there could be influences of other downstream signaling events following Gq activation.

Another important variable that needs to be considered when examining signaling in cardiac myocytes is the overall role of Ca^{2+} and electrical stimulation. Activated G-proteins interact with ion channels, transporters, and other signaling molecules located at the membrane that may contribute to in vivo effects. Within the contracting cardiomyocyte, there is a flux of Ca^{2+} into the cell initiating Ca^{2+} release from the sarcoplasmic reticulum and this rapid elevation of intracellular Ca^{2+} is needed for contraction of the myofilaments. Since some Gq-coupled receptors have been shown in some cell types to transactivate the EGFR in a Ca^{2+}-dependent manner[13], Ca^{2+} becomes a vital second messenger that may also contribute to effects similar to what Howes et al. report and contribute to specific components of Gq signaling. Of importance here is that this Ca^{2+}-dependent EGFR transactivation has not yet been shown in myocytes. However, Ca^{2+} is also important in heart disease since it can lead to the activation of Ca^{2+}-calmodulin kinase (CaMKII) and Ca^{2+}-dependent PKC isozymes and this can complicate downstream kinase signaling including Akt activation and directly assessing mechanisms.

Genetic approaches provide researchers with great tools in order to delineate signaling pathways and observe the effects of targeted molecules on the system chosen. Instead of using genetic approaches to study the effects of Gαq activation, a recent paper by Sabri et al.[14] describes use of an intracellular Gαq agonist (recombinant *Pasteurella multocida* toxin (rPMT)) to elucidate the role of endogenous Gαq signaling in NRVM. Activation of NRVM with rPMT revealed that novel isoforms of PKC (δ and ε) were activated by rPMT treatment but not the conventional PKC that is present in rat NRVM (PKCα). This paper highlighted that following exposure to rPMT (endogenous Gαq activation) for 24 hours, there is a decrease in the amount of phospho-Akt. There was also a small but insignificant increase in apoptosis measured by TUNEL-positive cells when exposed to H_2O_2, reinforcing the thought that cardioprotection is lost when the cells are activated with rPMT. Further the study went to show that there was decreased phospho-Akt when GF109203X (non-selective inhibitor of PKC α, δ, and ε) was used in rPMT treated cells implicating the novel PKC isoforms δ and ε in repression of Akt activation.[14] In the current manuscript by Howes et al., when the same inhibitor was used, there were no alterations in phospho-Akt [4]. Since Gαq signaling appears to be so reliant on the amount of expression, differences in Gαq levels could be responsible for the differences observed. Also, the abundance or translocation of PKC isoforms could have been different between the two studies. The physical location and compartmentalization of the signaling molecules must also be considered since this plays a vital role in their ability to activate other downstream molecules.

The differences in physiological versus pathological PI3K signaling could also be responsible for the differences between studies. A recent review by Dorn and Force[1] highlights the different isoforms of PI3K activated by the IP_3 pathway (γ) and that of the IGF-1 (α) pathway. The activation of PI3K is different between these two pathways as both rely on the p110 molecule for signaling but once again different isoforms are associated with the two pathways: p110γ for Gq coupled receptor and p110α for the growth factor receptors. Interestingly, p110α is responsible for physiological growth, and is not needed for pathological growth whereas p110γ is opposite in mediating pathological hypertrophy, but not exercise or physiological hypertrophy. Both PI3K

isoforms lead to Akt activation, however, it should be noted the Akt activation can lead to both physiologic and pathologic growth through GSK3β and/or mTOR. The balance between activation of Gαq and EGFR, which PI3K isoform is activated and signaling duration could all play a major role in the effects observed in the current paper.

Although Akt activation via Gαq could prove to be beneficial in treating disease, it must also be noted that inhibiting Gq signaling altogether has been shown to have beneficial effects in the heart by attenuating hypertrophy and maladaptation following pressure overload.[15, 16] This was done with a peptide inhibitor of GPCR-Gαq coupling and in transgenic hearts expressing this peptide inhibitor of Gq, hypertrophy was attenuated following transverse thoracic aorta constriction (TAC) and chronically this led to preservation of cardiac function and prevention of maladaptive remodeling.[16] Gαq inhibition has also proven to be successful in other tissues including vascular smooth muscle where Gαq inhibition attenuates hypertension induced by AngII.[17] Moreover, the success of clinical trials (LIFE[18] and EUROPA[19]) showing improvements in LV hypertrophy following treatment with Losartan (AngII receptor antagonist) and perindopril (ACE inhibitor) also cannot be ignored since these drugs do decrease overall Gq signaling in the cardiomyocyte. Thus, although Gαq stimulation can lead to cell survival, there is ample evidence that Gq inhibition is beneficial in HF

In summary, the current manuscript by Howes et al. in JMCC illustrates the ability of Gαq to directly activate two separate downstream pathways independently and initiate two different effects: hypertrophy and cell survival via Akt activation that is dependent on Src/EGFR/PI3K activation. The triggers that cause hearts in compensated hypertrophy to progress into a state of cardiac failure are still unknown. The current manuscript offers Gαq as a candidate that may play a role in mediating this balance between pathways. It also reminds us that multiple signaling pathways are continuously activated by any one molecule and that dissecting out the specific pathways involved mechanistically may provide novel targets for future HF therapies.

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