Structure, function, pharmacology, and therapeutic potential of the G protein, Goq,q,11

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INTRODUCTION

G protein coupled receptors (GPCRs) constitute the largest class of cell surface receptors. GPCR genes account for 5% of the human genome (1, 2). Of these receptors, all are seven membrane spanning receptors but not all are G protein binding but it is convenient to refer to the receptors as GPCRs. GPCRs also represent the largest and among the most efficacious class of therapeutic targets for diseases including cardiovascular disease, cancer, and asthma (1, 2). Many drugs have been developed based on GPCRs and these include some of the most important agents in human medicine, for example, in the treatment of asthma and hypertension (3). GPCRs are generally ligand activated but they can also bind to Goq-subunits in the absence of a ligand, a phenomenon known as receptor pre-coupling. GPCRs interact with their respective G proteins only upon receptor activation known as the collision coupling model or in the absence of agonist known as the pre-coupled receptor model (8).

Whereas protein tyrosine and serine/threonine kinase receptors have intrinsic catalytic activity, GPCRs do not have enzymatic activity per se but are linked to Goq proteins, which are GTPases, and mediate the signal transduction (9). G proteins of the α, β, and γ families provide the specificity and functionality of GPCRs.

G proteins are classified into four families according to their α subunit: Gαi, Gαs, Gα12/13, and Gαq. There are several downstream pathways of Gαq of which the best known is upon activation via guanosine triphosphate (GTP), Gαq activates phospholipase Cβ, hydrolyzing phosphatidylinositol 4,5-biphosphate into diacylglycerol and inositol triphosphate and activating protein kinase C and increasing calcium efflux from the endoplasmic reticulum. Although G proteins, in particular, the Gαq/11 are central elements in GPCR signaling, their actual roles have not yet been thoroughly investigated. The lack of research of the role on Gαq/11 in cell biology is partially due to the obscure nature of the available pharmacological agents. YM-254890 is the most useful Gαq-selective inhibitor with antiplatelet, antithrombotic, and thrombolytic effects. YM-254890 inhibits Gαq signaling pathways by preventing the exchange of guanosine diphosphate for GTP. UBO-QIC is a structurally similar compound to YM-254890, which can inhibit platelet aggregation and cause vasorelaxation in rats. Many agents are available for the study of signaling downstream of Gαq/11. The role of G proteins could potentially represent a novel therapeutic target. This review will explore the range of pharmacological and molecular tools available for the study of the role of Gαq/11 in GPCR signaling.

Keywords: G proteins, GPCR, cell signaling, therapeutic targets, transactivation

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www.frontiersin.org
March 2015 | Volume 2 | Article 14 | 1
Kamato et al. Pharmacology and therapeutic potential of Ga/q,11

FIGURE 1 | Classification of G proteins into four families according to their α subunit. The Ga subunit is made up of Gas, Gai, Gaq/11, and Ga12/13. Gas and Gai families regulate adenylyl cyclase activity, while Gaq activates PLC-β and the Gu12/13 can activate small GTPase families. The Gaq subunit is made up of four members, which include the Gaq, Gaq/11, Gaq/14, and Gaq/15/16.

the Chromobacterium sp. Initial studies indicated that this is a specific inhibitor of Gaq/11. YM-254890 has had variable availability and has not been available in recent times. As the importance of GPCR signaling in physiology and pathophysiology continues to grow, the potential importance of G proteins increases both for the fundamental cell biology and as potential therapeutic targets.

One of the major and expanding areas of GPCR signaling is transactivation-dependent signaling (13) in which GPCRs transactivate protein tyrosine kinase (PTK) and protein serine/threonine kinase receptors (14–16). Transactivation greatly expands the roles of GPCRs in cell biology (13, 17–19). GPCR transactivation of PTK receptors was discovered in 1996, has been the subject of almost 200 publications, and has been recently reviewed (20). Our laboratory has recently extended the paradigm of GPCR to PTK receptor transactivation to include the transactivation of protein serine/threonine kinase receptors (14–16). Transactivation greatly expands the roles of GPCRs in cell biology (13, 17–19). GPCR transactivation of PTK receptors was discovered in 1996, has been the subject of almost 200 publications, and has been recently reviewed (20). Our laboratory has recently extended the paradigm of GPCR to PTK receptor transactivation to include the transactivation of protein serine/threonine kinase receptors and specifically the protease-activated receptor (PAR)-1 and endothelin receptor (ETR)-mediated transactivation of the transforming growth factor (TGF)-β type I receptor (TGFR1) also known as Activin-like Kinase (Alk)-V (15, 16, 21). There is very little information on the role of Ga proteins in GPCR transactivation signaling. There is a need for synthetic programs to provide new molecules with the pharmacological properties of YM-254890 and such programs will provide agents, which allow for a much broader range of studies on the role of G proteins in GPCR signaling. This review focuses on the role of Gaq/11 in GPCR signaling in the context that the availability of new tools will shortly lead to a large increase in studies in this area. The two targets of compound such as YM-254890 are Gaq and Ga11 – these two proteins are distinct gene products but they have an identical number of amino acids and essentially identical structures and functions. In this review, we refer to Gaq but most statements will also relate to Ga11 and only where differences are known and of significance will this distinction be drawn.

Gαq/11 SIGNALING

The responses to GPCR agonists and the conformational changes in the GPCR that are induced by ligand binding are transduced and then mediated by heterotrimeric G protein complexes. Consisting of three subunits α, β, and γ, their role is to transduce external stimuli into intracellular signaling cascades. Most of the specificity of signaling resides in the Ga subunit. In an inactivated state, the α subunit binds guanosine diphosphate (GDP); however, upon binding activation of the GPCR, GTPase activity is induced and promotes the exchange of bound GDP for guanosine triphosphate (GTP). The α subunit and βγ complex then dissociate from one another and interact with their associated effectors (22). In the most common signaling pathways, Gaq activates phospholipase Cβ (PLCβ), which hydrolyzes phosphatidylinositol
4,5-bisphosphate (PIP₂) releasing diacylglycerol (DAG) and 1,4,5-
inositol trisphosphate (IP₃). DAG activates a number of isoforms of
protein kinase C (PKC), whereas IP₃ diffuses to the endoplasmic
reticulum (ER) and binds to IP₃ receptors on ligand-gated calcium
channels on the surface of the ER leading to a massive release of
calcium ions into the cytosol and subsequently in some cells, the
opening of cell surface calcium channels leading to the influx of
extracellular calcium (23). The calcium cycle continues with the
uptake of calcium back into the ER by Ca ATPases.

In addition to this paradigm, it has been shown that RhoA is
a mediator of calcium sensitization and is downstream of Gaα
signaling. Activation of the members of the Rho family is via
GTP binding. The exchange of GDP for GTP on these proteins is
controlled through guanine nucleotide exchange factors (GEFs),
which catalyze the exchange of GDP for GTP (24). Activation of
RhoA is only when RhoA is active that it can interact with and activate
a G protein coupling the exchange of GDP for GTP (25). It is
suggested that the GEFs utilized may involve P2Y-1 receptors on ligand-gated calcium
three switch regions, which are flexible loops that change
conformation when bound with GTP. The helical domain contains
six α-helices, which encapsulates nucleotides in the protein core
by forming a lid over the nucleotide-binding pocket. Of all G pro-
tein families identified, members of the Gaq family share the most
amino acid sequence homology. In humans, Gaq11, Gaq14, and Gaq16
share 90, 80, and 57% sequence similarities, respectively (27).

FUNCTIONS OF Gaq

Gαq plays a role in platelet aggregation. Bleeding time and
resistance to thromboembolism are dramatically increased in
Gaq-deficient mice compared to wild type (28). Gaq is also
implicated in insulin-stimulated glucose transport (29). In 3T3–
L1 adipocytes, Gaq is required for insulin-induced GLUT4
translocation and the stimulation of 2-deoxy-D-glucose uptake.
Angiotensin II dose-dependently increases cell proliferation in
smooth muscle cells and this is inhibited by the Gaq antago-
nist, GP2A (30). Gaq11 proteins are involved in HIV-1 envelope
glycoprotein-dependent cell–cell fusion upstream of Rac-1 (31).
Genetically modified mice studies suggest that receptors coupled
to the Gaq play a role in the transactivation of kinase
pathway. The molecular mechanism of Gaq11 induced apoptosis
leading to the activation of Rho/ROCK is not clearly understood;
however, some studies have shown that Gaq11 signaling activated
RhoA, which inhibited insulin-stimulated Akt phosphorylation in
HeLa cells. In CHO cells, Gaq acts as an activator of cytoskele-
ton remodeling through the activation of ADP-ribosylation factor
6. Platelets stimulated with P2Y1 agonist leads to the activation
of RhoA, this activation was inhibited by Gaq inhibitor YM-
258490, indicating that RhoA activation downstream of purinergic
(P2Y)-1 receptors requires Gaq stimulation (26).

ROLE OF Gaq IN THE GPCR TRANSCACTIVATION OF KINASE

There are now two major pathways of GPCR to cell surface recep-
tor kinase transactivation – the well-established transactivation of
PTK receptors, notably epidermal growth factor receptor (EGFR)
and the recently identified transactivation of serine/threonine
kinase receptors, specifically the TGFBR1 (14–16, 33). There
is some information of the role of Gaq proteins and thus Gaq in
the transactivation of PTK receptors but nothing is known of
the role of Gaq in the transactivation of serine/threonine kinase
receptors.

G protein coupled receptors coupled to Gaαq, such as bombesin
receptor or Gaαq, proteins, such as M2 muscarinic acetylcholine
receptor, expressed in COS-7 cells show increased EGFR tyro-
sine phosphorylation more than that resulting from Gaq coupled
receptor stimulation. Cells transfected with Gaqαq-coupled GPCRs
are unaffected by pertussis toxin while Gaqαq coupled receptors
are, as expected, blocked by pertussis toxin treatment (34). Thus,
EGFR transactivation may occur through both pertussis toxin-
sensitive and -insensitive pathways. GPCR transactivation of ser-
ine/threonine kinase receptors and specifically TGFBR1 by both
ETR and PAR-1 has been identified in vascular smooth muscle
cells (VSMCs) but the role of Gaq in transactivation of TGFBR1
has not been reported (15, 16, 21).

STRUCTURE OF Gaq

Gαq and Gα11 are distinct gene products but from the same chro-
mosome (12). These two proteins have an identical number of
amino acids and are functionally almost identical. However, the
tissue distribution of the two isoforms is distinct (12). Gaq is a 359

www.frontiersin.org
March 2015 | Volume 2 | Article 14 | 3

Thus far, the biochemical mechanisms of GPCR to protein tyrosine and protein serine/threonine kinase receptors have been found to be completely distinct with, for example, the former involving MMPs and the latter being independent of MMPs (16). The transactivation of serine/threonine kinase but not tyrosine kinases involves the cytoskeleton (16). The independent signaling pathways have made it difficult to envisage a single potential therapeutic target for the inhibition of all GPCR transactivation signaling (18).

It will be interesting to investigate the role of Gαq proteins in tyrosine and serine/threonine kinase transactivation signaling as it has the potential to be a point of commonality in GPCR-mediated transactivation of cell surface protein tyrosine and serine/threonine kinase receptor signaling.

MOLECULAR AND PHARMACOLOGICAL REGULATION OF G PROTEINS

G proteins in cells can be effectively knocked down utilizing a molecular approach and this has allowed for detailed studies of the function of various G proteins and their interactions. Classic experimental approaches assume that the intervention is specific and does not alter other parameters that would impact on the experimental result of the index intervention. This is not always the situation and is certainly not the reliable paradigm in the case of the regulation of G proteins. Gilman and colleagues (35) demonstrated that knocking down Gβ proteins resulting in a compensatory increase in both the effector, adenylyl cyclase and even the GPCR, being the β2-adrenergic receptor. Results of knock down interventions are also not always reciprocal – the knock down of one G protein may lead to a compensatory increase in another G protein family member but the reverse or reciprocal phenomenon may not occur (35). Thus, the knock down of Gαq and Gα11 in HeLa cells increased the accumulation of Gαq and Gαq but the reciprocal response did not occur (35).

Gα and β proteins exist in approximately equal mass stoichiometry in most cells. This occurs primarily because Gβ proteins stabilize bound Gα proteins with the corollary that free Gα proteins are degraded. However, Gα proteins are subject to palmitoylation and myristoylation and these processes may bind Gα proteins to the cell membrane and stabilize the proteins (36). A consequence of the role of post-translational regulation on stability and the cellular levels of G proteins is that the relationship between mRNA and protein levels may be perturbed. Higher mRNA levels may lead to increased expression of the G protein, but if it is orphaned and free the protein may be degraded providing for high level of mRNA and in the presence of low levels of protein.

Molecular approaches to the up- and down-regulation of target proteins are a major component of modern mechanistic studies of cell biology. However, as exemplified above, alteration of target protein levels may result in compensatory changes in other components of a system and the perturbation might not provide the expected result. Pharmacological approaches nullify the activity or function of a target protein without in most cases altering the level of the target protein. If there is greater availability of G protein inhibitors such as YM-254890 or alternative new tools, then it will be interesting to determine if blocking a Gα protein results in any changes in the level of other G proteins within the cell. Such studies are currently underway in our laboratory.

PHARMACOLOGY OF Gαq INHIBITORS

YM-254890

The compound known as YM-254890, a cyclic depsipeptide isolated from the Chromobacterium sp. QS3666, is a specific Gαq inhibitor. YM-254890 has been shown to inhibit ADP-induced platelet aggregation, which is mediated via GPCRs, P2Y1, and P2Y12 (37). These receptors are associated with the Gαq and Gα11 signaling pathways, respectively. YM-254890 has no effect on the P2Y12 signal transduction pathway, indicating that the compound has some specificity for Gαq. It was also shown to inhibit Gαq-coupled GPCR signaling by inhibiting calcium mobilization in P2Y2-expressing C6-15 cells but not cAMP accumulation (38).

YM-254890 inhibits the signal transduction of Gαq by inhibiting the exchange of GDP for GTP preventing the activation of the G protein, rather than receptor-Gαq interactions (38). When bound to GDP, the non-polar side chains of YM-254890 form hydrogen bonds with the Switch I region; however, this is a conformation that cannot be maintained when bound with GTP (39). Aside from antiplatelet activity, by electrically inducing carotid artery thrombosis in rodents, YM-254890 was also shown to have antithrombotic and thrombolytic effects (40).

YM-254890 was discovered and developed by Yamaguichi Pharmaceuticals, Japan; Yamaguichi subsequently became the property of Astellas Pharmaceuticals, Japan. YM-254890 was made available to researchers 10 years ago and a small number of interesting studies were published. The initial results indicated that YM-254890 is a useful tool for investigating Gαq11-coupled receptor signaling and the physiological roles of Gαq11. For example, Gαq knock-out mice have lower blood pressure than appropriate controls (41). This indicates some potential for a Gαq inhibitor to be an anti-hypertensive agent and accordingly YM-254890 has not been provided to researchers presumably because of such identified commercial value. As discussed above, molecular approaches in this area, for example, G protein knock down can lead to rebound increases in other G proteins with unexpected results. Accordingly, it is understood that a number of groups are undertaking programs for the synthesis of compounds related to YM-254890 and it is likely that the availability of potent and specific Gαq11 inhibitors would greatly expand activity and knowledge in this area and answer important questions such as the role of Gαq11 in GPCR transactivation signaling of protein kinase receptors.

UBO-QIC/FR300359

FR300359, henceforth referred to as UBO-QIC, is also, like YM-254890 a cyclic depsipeptide; it is isolated from the Ardisia crenata sims plant (42). UBO-QIC is structurally very similar to YM-254890 and not surprisingly shows similar pharmacological activity. UBO-QIC inhibits platelet aggregation in rabbits in vitro and causes dose-related hypotension in anesthetized normotensive rodents, which is consistent with the effect on blood pressure in Gαq knock down mice (41, 43). The blood pressure lowering effect was attributed to the ability of UBO-QIC to partially mediate nitric oxide release from endothelial cells and inhibit calcium migration caused by voltage-dependent and receptor-operated channels (44).
Since the discovery of UBO-QIC as a G\(\alpha_q\) antagonist, there have been limited studies showing its use. In HEK cells transfected with TRPV4, PAR-2-mediated intracellular calcium release was abolished by UBO-QIC when compared to control; however, extracellular calcium influx through the TRPV4 ion channel was unaffected thus showing that PAR-2 coupling to TRPV4 is not mediated by G\(\alpha_q\) signaling (45). There have been no studies directly comparing the activity of YM-254890 and UBO-QIC possibly because of the linked variability of the former compound whereas at the time of preparing this review, UBO–QIC is commercially available.

THE PEPTIDE ANTAGONIST GP-2A
In 2004, Tanski et al. (30) discovered a competitive G\(\alpha_q\) inhibitor, G Protein antagonist-2A, also known as GP-2A. GP-2A is a peptide that selectively inhibits the action of G\(\alpha_q\) by M1 muscarinic cholinergic receptors. The signaling pathway of G\(\alpha_q\) and its role in cell proliferation with rat pulmonary artery smooth muscle cells were studied. Angiotensin II-mediated proliferation, PLC\(\beta\) activation, and Erk1/2 phosphorylation were inhibited by more than 50% in the presence of GP-2A (30). The EGFR can be activated by EGF to generate an intracellular signaling pathway leading to the phosphorylation of several downstream effector proteins such as Erk1/2 (46). Tanski and colleagues have evaluated angiotensin II (as specific G\(\alpha_q\) agonist) to effectively reduce Erk1/2 activation mediated by PLC\(\beta\) via G\(\alpha_q\) in the presence of GP-2A by showing its association with the phosphorylation of Erk1/2 in rat pulmonary artery smooth muscle cells (30). This study provides a strong foundation for our laboratory research as we can further investigate the possibility of this downstream signaling pathway to see whether or not GP-2A can act on other GPCR agonists such as thrombin to effectively respond similarly via G\(\alpha_q\) in other smooth muscle cell types such as human VSMCs.

OTHER PHARMACOLOGICAL TOOLS FOR EVALUATING THE ROLE OF G\(\alpha_q\) IN GPCR SIGNALING
It is also possible to indirectly assess the role of G\(\alpha_q\) in GPCR signaling by analyzing downstream events through the use of inhibitors (Figure 2). For example, as detailed above GPCR ligand engagement activates G\(\alpha_q\) which in turn activates phospholipase C leading to the catalysis of PIP\(_2\) and the release of DAG and IP\(_3\). There are inhibitors of PLC–\(\beta\) including U73122, and its inactive analog U-73343 is available to use as a control compound. These compounds have been widely used (47–51) although they are not considered to be especially useful and specific agents. The antibiotic, neomycin, can also be used as a PLC\(\beta\) inhibitor in that it binds to the target substrate, PIP\(_2\) and inhibits the action of PLC\(\beta\) to release DAG and IP\(_3\), which can be assessed as a reduction in IP\(_3\) accumulation or increased free intracellular calcium (23). As always with pharmacological approaches, it is likely that the use of multiple approaches can provide the best information on the role of G\(\alpha_q\) in GPCR signaling (Table 1).

U73122 AND ITS INACTIVE ANALOG U-73343
To maximize our knowledge of G\(\alpha_q\), it is possible to examine the downstream role of G\(\alpha_q\) in GPCR by assessing the inhibitors of PLC, U73122, and its inactive analog U73343. U73122 and its analog U73343 were used to show the effect of human platelet

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**FIGURE 2** | Pharmacological agents to inhibit downstream signaling intermedated of G\(\alpha_q\). Once GPCR is activated by its agonist, G\(\alpha_q\) signaling activates phospholipase C \(\beta\) (PLC\(\beta\)), which leads to the hydrolysis of phosphatidylinositol 4,5-biphosphate (PIP\(_2\)) and diacylglycerol (DAG). The former leads to the release of 1,4,5-inositol tris phosphate (IP\(_3\)), initiating calcium release, activating protein tyrosine kinase 2 (PYK2), which leads to proto-oncogene tyrosine protein kinase (Src) activating Ras guanine nucleotide exchange factor (Ras GEF), which leads to the activation of MAPK signaling. MAPK signaling pathway can also be downstream of DAG that activates protein kinase C (PKC), which leads to the activation of MAPK signaling. G\(\alpha_q\) signaling can also go indirect of PLC\(\beta\) by activating Rho GEF leading to the activation of the Rho/ROCK signaling pathway.
Table 1 | Pharmacological tools used as inhibitors of Goq or its downstream signaling intermediates.

| Inhibitors       | Actions                        | Reference |
|------------------|--------------------------------|-----------|
| Direct           |                                 |           |
| YM-254890        | Goq inhibitor by inhibiting the exchange of GDP for GTP | (42)      |
| UBO-QIC          | preventing the activation of the G protein | (37)      |
| GP-2A            | Competitive Goq inhibitor       | (30)      |
| Downstream       |                                 |           |
| U73122/ U73343   | PLC-β inhibitors                | (47)      |
| Neomycin         | Binds to PIP2 and blocks the action of PLC-β | (23)      |
| BAPTA-AM         | A chelator of calcium ions      | (52)      |
| Y-27632          | Reversing increase in intracellular calcium | (63)      |

calcium signaling and protein tyrosine phosphorylation in the presence of thrombin, collagen, and thapsigargin (47). U73122 showed complete inhibition of calcium signaling in the presence of this agonist, which was generated via the activation of PLC specifically the β and γ isoforms (47, 54). U73343 did not show any calcium inhibitory effect via the activation of PLC but rather showed the calcium inhibitory effect via the upstream activation of cPLA2 in the presence of thapsigargin and collagen (47). This provides a clear indication that U73343 has minimal activity as a PLC inhibitor.

The study also investigated the role of Goq in the transactivation of PTK receptors to show that platelets, stimulated by thrombin increased protein tyrosine phosphorylation. In the presence of U73122, the phosphorylation of tyrosine kinase was abolished (47). As mentioned earlier, Goq are prominent in signaling in VSMCs. From the recent study, it is possible that we can replicate this investigation in other cell type to further study the PTK and perhaps the serine/threonine kinase transactivation pathway in the presence of U73122.

**NEOMYCIN AND ITS POTENTIAL ROLE FOR Goq SIGNALING STUDIES**

As mentioned above, another agent, which can further be investigated, is neomycin, a PLC inhibitor. Our laboratory has previously reported that neomycin strongly inhibits the formation of IP3 in rat aortic smooth muscle cells in the presence of endothelin, an agonist that influences contraction in smooth muscle (23). Endothelin acts via specific ET-A receptors, which are coupled to PLC to stimulate calcium mobilization (23). ETR is coupled to PLC via G proteins (55) and its activation acts on the cardiac muscle where it binds to ryanodine located on the SR, which releases calcium mobilization within the cardiac muscle (56). As known, the signaling pathways of GPCRs through G proteins contribute to various functions in different cell types such as the contraction of blood vessels and are involved in many diseases such as cancer and cardiovascular disease (16, 57). In unpublished data, we have found that neomycin has a dose-dependent inhibition of thrombin-mediated release of intracellular calcium in human VSMCs. Further investigation is required to understand its pharmacological signaling pathway via Goq.

**THE ACTION OF INTRACELLULAR CALCIUM ION TO STUDY THE ROLE OF Goq IN GPCR SIGNALING**

The activities of calcium ions on the inhibitory action of calcium channel blockers and its impact on atherogenesis in the regulation of proteoglycan biosynthesis in human VSMCs was studied via the role of ionomycin and bis (2-aminophenyl) ethlyleneglycol-N,N,N′,N″-tetraacetic acid, tetraacetoxymethyl ester (BAPTA-AM) (58).

Ionomycin is a calcium ionophore, which elevates intracellular calcium (58). Radioactive sulfate incorporation into proteoglycans was unaffected by ionomycin, providing support that calcium regulation is not involved in proteoglycan synthesis (58).

Similarly, BAPTA-AM, a chelator of calcium ions, which prevents an elevation of intracellular calcium by acting as a calcium buffer (52) had no effect on proteoglycan synthesis (58). Agonists, TGF-β, and ET-1 stimulated BAPTA-AM to decrease sulfate incorporation into proteoglycans. The interpretation therefore concluded that there were no effects on calcium ion stimulation hence intracellular calcium does not play a role in VSMC proteoglycan synthesis (58).

**Y-27632 – A RhoA/ROCK INHIBITOR**

Y-27632 is a widely used specific inhibitor of RhoA/ROCK family of protein kinases (53). The ROCK family of kinases is involved in Rho-induced formation of actin-stress fibers and focal adhesion as well as the down-regulation of myosin light chain (MLC) phosphatases. Deng et al. (59) examined the role of the PLC calcium pathway and Rho Kinase in PAR-1-mediated CCL2 release. Rho kinase activation is mediated by a Goq-PLC-calcium-dependent PKC pathway to release thrombin-mediated CCL2. Thrombin-induced phosphorylation of MLC was inhibited by PLC calcium and calcium-dependent PKC inhibitors. Q94 a PAR-1 selective Goq antagonist abolished thrombin-mediated MLC phosphorylation. Subsequent experiments showed that blockade of Rho kinase signaling is not essential for CCL2 protein production but is important in the release of CCL2 from the cell, as thrombin-mediated CCL2 levels are inhibited by Y-27632.

Having provided evidence that cytoskeletal rearrangement is involved in thrombin-mediated transactivation of TGFBR1, ROCK inhibitor Y-27632 was used to study the role of ROCK in the transactivation signaling pathway. Y-27632 inhibited the downstream product of ROCK-phospho-Ezrin, Radixin, and Moeisin. The ROCK inhibitor abolished the thrombin-mediated increase in phosphoSmad2, indicating that Y-27632 inhibits the activity of ROCK and ROCK is involved in the thrombin-mediated transactivation of TGFBR1. To evaluate the role of ROCK signaling in cardiac contractility, hearts were treated with Y-27632. This lead to a significant inhibition in the peak of pressure of non-transfected hearts but no reduction in basal contractility in hearts overexpressed with a 1A-adrenergic receptor signifying that ROCK pathways play an important physiological role in maintaining baseline contractility (60).
**Gα GENE KNOCKDOWN USING siRNA**

Despite the very large number of GPCRs, there are relatively few studies that have used the potential of Gq/11 gene knockdown by siRNA to explore their roles in the signaling cascades. One of the first reported gene knockdown studies of Gα proteins was the knockdown of Gq and G11 gene expression using siRNA in HeLa cells (35). This work demonstrated an absolute requirement of Gq/11 to stimulate histamine-mediated phospholipase C activity. Silencing of Gq or G11 caused indistinguishable phenotypes, loss of half of histamine-stimulated PLC activity, despite the fact that concentrations of Gα11 exceeded those of Gq by 10-fold. No compensatory increases of either Gq or G11 were observed following loss of either protein. Loss of Gq or G11 did cause increased accumulation of Gαi and Gαo (35). A study characterizing the Gα subunits required for PAR-1-mediated endothelial cell permeability showed that both Gq and G11 were necessary for thrombin to increase permeability while the need for Gα12/13 was less. Both protein subunit families contributed significantly to RhoA activation by thrombin (61). Knockdown of Gq/11 in human pulmonary artery smooth muscle cells alters but does not prevent hypoxia-induced mitogenic factor-mediated calcium release demonstrating that Gq/11 contributes to hypoxia-induced PLC signaling pathway (62). Using siRNA knockdown of Gq or G11 in human prostate epithelial cells, GPCR melatonin receptor gene knockdown using siRNA while overexpression of Gq/11 has been to co-express Gα protein mutants or chimeras in different cell types. Gq/11 chimeric mutants containing Gαq or Gαo tails co-expressed in COS-7 cells with opioid receptors and stimulated with opioid agonist are insensitive to pertussis toxin catalyzed ADP-ribosylation demonstrating an inability of Gq or Gαo tails to serve as pertussis toxin substrates (70). Gi-coupled opioid receptors increase Gq signals as demonstrated by the co-expression of constitutively active Gα mutants in COS-7 cells and requires activated phospholipase beta and Gβγ dimers (71).

A considerable number of studies have explored membrane localization of Gα proteins in different cellular contexts and described a diversity of requirements. N-terminal sequence-mutated Gα proteins expressed in HEK293 cells are unable to localize to the plasma membrane due to their inability to bind to Gβγ or attach myristate and palmitate (72). Mutated Gq and Gαo proteins deficient in Gβγ-binding and co-expressed with different β1–5 or γ2/3 subunits show that Gβγ and Gαo proteins promote membrane localization of the other and requires palmitoylation (73). Defects in plasma membrane localization of Gq occur when four N-terminal basic residues are mutated to glutamine; however, mutation of nine basic residues in Gq4 is required. Gβγ co-expression partially rescues localization indicating that the characteristics of the N-terminal residues of Gαo and Gq4 are critical in membrane localization of these proteins (74). Using co-expressed constitutively active Gq4 or Gα12, the activation of ETRs was shown to mediate the binding of Gq4 or Gα12 in caveolae to enable the downstream activation of Erk1/2 (75).

Gα protein-mediated signaling studies across a wide variety of GPCRs dominate the literature using Gα protein mutants or chimeras. Constitutively active Gq4, Gq12, or Gq13 mutants transfected into Jurkat cells co-expressing GPCR muscarinic cholinergic receptor subtypes demonstrated a requirement for Gq13 to activate downstream transcription factor serum response factor. However, the M1 subtype also required Gq11 and calcium when regulator proteins RGS2 and RGS4 were co-transfected that demonstrates a unique pathway for the M1 receptor (76). Gq11 (Y356D) mutation results in altered GPCR α1B-adrenergic receptor contact domain and abolishes receptor function, however, does not affect ligand binding (67). Studies using constitutively active mutants Gq4 (Q209L) and Gq13 (Q226L) demonstrate that Gq4 activates rat brain phospholipase D1; however, Gq13 inhibits its activity (77). Gq deletion mutants were used to demonstrate that Gq mediates down-regulation of the vesicle-associated GPCR vesicular monoamine transmitter transporter VMAT2 activity in

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**Kamato et al. Pharmacology and therapeutic potential of Gαq/11**

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www.frontiersin.org March 2015 | Volume 2 | Article 14 | 7
platelets (78). Expression of a constitutively active Goq (R183C) mutant inhibited the expression of ezrin–radixin–moesin-binding phosphophosphoprotein 50 and subsequent internalization of GPCR thromboxane A(2) beta receptor independently of PLC and PKC pathways (79). Specific Gα peptides and dominant negative Gα mutants were used to demonstrate the ability of α-thrombin to activate different effectors via Gαq, Gβγ, and Goα2, respectively, in Chinese hamster embryonic fibroblasts and thereby regulate the activation of the PI3Kinase/Akt pathway (80). Molecular modeling and testing GST–fusion proteins of Goq mutants–GPCR kinase complexes revealed a critical residue Goq Pro185 at the interface with GPCR kinase 2 with residues Goq K77, L78, Q81, and R92 also playing key interactive roles (81). Constitutively active Goqα, Goqα/12, and Goqα/13 overexpressed in human astrocytoma cells increased agonist-activated thromboxane A2 receptor-mediated IL-6 production while mutated Goqα and Goqα/13 overexpression blocks IL-6 production (82). Using both constitutively active and dominant negative Goqα subunit expression showed that in neureloblastoma cells Goqα elicits a rapid signal at the plasma membrane (83). Expression of constitutively active Goqα (Q209L) mutant inhibits Ras and the PI3K/Akt pathway; however, minimal effects are seen on the Ras/Raf/MEK/Erk signaling pathway (84). Goqα mutants that cannot bind Gβγ are unable to be stimulated by the mitogenic Pasteurelosis multocida toxin (PMT) demonstrating the requirement of cohesive Goqα/Gβγ signaling for this toxin activation pathway (85). Expressing GTPase-deficient Goqα mutant in the human adrenal cell line H295R depolarizes the two-pore loop potassium channel TASK and thereby increases aldosterone secretion (86). Chimeric G proteins have been used to determine Gα responses from orphanGPCRs with unknown Gα coupling partners. A luciferase reporter system with a chimera that contains promoter elements that drive Gα, Gq, and G12 signals and another chimera with promoters to drive Gα signals revealed neuromedin U receptor 1 activating Gq, neuromedin U receptor 2 activating Gi, and luteinizing hormone receptor activating Gq and Gα proteins (87).

**POSSIBLE OF Goqα AS A THERAPEUTIC TARGET**

Goqα as a protein has several functions, which are valuable therapeutically. The GTPase activity, which hydrolyzes bound GTP to GDP, is an enzyme action that can be targeted. The binding of GDP and GTP are potential targets in the same manner in which the ATP binding site is target of many drugs inhibiting kinases (88). The ligand-activated GPCR acts as a GEF, which stimulates the exchange of GDP for GTP on the Gα peptide and this could be targeted. Furthermore, the protein contains a switch mechanism and this can be targeted as it is the target of the YM class of inhibitors (39). So, it is both theoretically possible and has been demonstrated that Goqα can be exploited as a drug target.

The consequences of targeting signaling molecules have theoretical limitations based on the role of such targets in normal physiology but also conceptually there may be situations, pathophysiology, in which the activity of Goqα is elevated or enhanced and presents itself as a target. Such situations are common in therapeutics but in most cases can only be established experimentally.

As discussed above, inhibition of Goαq11 using YM-254890 has demonstrated anti-platelet aggregation, anti-thrombotic, and thrombolytic properties in a rat model of carotid artery thrombosis (40). Therefore, compounds that inhibit Goαq11 could show enormous potential in the treatment of thrombotic conditions such as thrombotic stroke and myocardial infarction in humans. Additionally, a number of recent studies have also implicated a role for Goαq11 in a range of metabolic conditions such as obesity and type 2 diabetes (89, 90). Activation of Goαq results in pronounced increases in blood glucose levels in a mouse model (89), thus, compounds that inhibit Goαq could also show promise as a future treatment option for type 2 diabetes.

In the important cardiovascular context of hypertension, Goαq knockout mice have reduced blood pressure (41) and YM-254890 has demonstrated some anti-hypertensive properties (40). Although there are many effective anti-hypertensive agents currently available, there are also many subjects with medication-resistant hypertension, which may require newer therapies although it is unclear if a Goαq inhibitor would be suitable to consider for such a niche.

**CONCLUSION**

G protein coupled receptor signaling is a major area of cell biology and therapeutics. The functioning of the seven transmembrane GPCR has been one of the most intensively studied areas of protein function. GPCRs signal through G proteins of the α and βγ subtypes where most of the signaling specificity is determined by the Gα protein. For the Gαq family protein, these signaling pathways include the well-known PLC, PKC, and IP3 pathways and the lesser appreciated Rho/ROCK pathway. For multiple reasons, mostly the limited availability of pharmacological agents, which inhibit G protein function, the role of G proteins in GPCR signaling has been severely under-studied relative to the intense activity around the GPCRs. This is true for the Gqα proteins, which are the subject of this review but also for other G proteins. Given the broad involvement of GPCRs in cellular functioning, this is a major deficit in cellular signaling studies and potentially more importantly in the search for new drug targets. The recently expanding area of GPCR signaling is that of transactivation-dependent signaling in which GPCR transactivation of protein tyrosine and protein serine/threonine kinase cell surface receptors enormously expands the range of activities associated with the respective GPCRs. The potential role of G proteins and Goq proteins in particular in GPCR transactivation signaling is one very interesting area to be explored. It is likely that there are programs of chemical synthesis underway to synthesize inhibitors of Goq proteins and these will increase the availability of inhibitors and also with the importance of this area hopefully lead to new studies, which produce a range of agents, some of which may be useful in in vivo studies. It is hoped that such studies may provide insights into the potential role of Goqα in disease processes and reveal the extent to which such inhibitors may represent novel therapeutic agents in a range of conditions from cancer to cardiovascular disease.

**AUTHOR CONTRIBUTIONS**

PL, NO, VC, and WZ conceived the focus of the review, wrote, and edited the paper. HK and MB provided chemical insight about...
cyclic depsipeptide. DK, LI, and RB contributed expertise with preparation of the manuscript and the figures.

ACKNOWLEDGMENTS

This work was supported by National Health and Medical Research Council Project Grant 2012–2014 (#1022800) (PL and NO). We thank the Ministry of Foreign Experts of the Government of the People’s Republic of China for support by way of a High End Professor (Education) Award through Zhongshan (Sun Yat-sen) University (WZ).

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 03 December 2014; accepted: 11 March 2015; published online: 24 March 2015.

Citation: Kamato D, Thach L, Bernard R, Chan V, Zheng W, Kaur H, Brimble M, Osman N and Little PJ (2015) Structure, function, pharmacology, and therapeutic potential of the G protein, Galpha(q,11). Front. Cardiovasc. Med. 2:14. doi:10.3389/fcvm.2015.00014

This article was submitted to Cardiovascular Therapeutics, a section of the journal Frontiers in Cardiovascular Medicine.

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