Chapter 13
G-Proteins in Vascular Complications of Diabetes

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Abstract Vascular complications including impaired contractility and increased cell proliferation are the most common complications associated with diabetes, and chronic hyperglycemia appears to be an important contributing factor in this process. However, the precise mechanism(s) responsible for hyperglycemia-induced vascular dysfunction remains poorly characterized. Guanine nucleotide regulatory proteins (G-proteins) play a key role in the regulation of various signal transduction systems including adenylyl cyclase/cAMP and phospholipase C (PLC)/phosphatidyl inositol turnover (PI) which are implicated in the regulation of a variety of vascular functions including cell proliferation, hypertrophy, vascular tone and reactivity and the aberration of these pathways contribute to vascular complications in diabetes. The levels of inhibitory G-proteins (Giα-2 and Giα-3) are decreased in several tissues from streptozotocin diabetic rats and diabetic subjects. A relationship between the development of diabetes and Giα protein expression is also shown and suggests a role of decreased levels of Giα proteins in the pathogenesis of diabetes. In addition, exposure of aorta as well as VSMC with high glucose that simulate diabetic state also decreased the levels of Giα-2 and Giα-3 proteins. A correlation between the levels of glucose (in vivo and in vitro) and decreased expression of Giα proteins exists and suggests that hyperglycemia may be a contributing factor in diabetes-induced decreased expression of Giα proteins. The decreased levels of Giα proteins and associated adenylyl cyclase signaling in diabetes/hyperglycemia are attributed to the enhanced levels of vasoactive peptides. In addition, hyperglycemia-induced enhanced nitroxidative stress also contributes to the decreased expression of Giα proteins induced by high glucose. Furthermore, the basal adenylyl cyclase activity and cAMP levels are decreased in VSMC exposed to high glucose. On the other hand, Gqα proteins and the downstream molecules PKC and DAG are upregulated in different tissues from STZ-induced diabetic rats. In addition, VSMC exposed to high glucose also have enhanced expression of Gq/11α, PLCβ-1 and PLCβ-2 proteins. The enhanced levels of vasoactive peptides induced by

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hyperglycemia through oxidative stress, c-Src, growth factor receptor activation and MAP kinase signaling contribute to the enhanced expression of Gqα, PLCβ proteins in VSMC. The enhanced expression of Gqα and PLCβ has been shown to contribute to VSMC hypertrophy. It is thus suggested that the decreased levels of cAMP, Giα proteins and as well as overexpression of Gqα and PLCβ may be the contributing factors responsible for the vascular complications of diabetes/hyperglycemia. This review briefly summarizes some of the key studies on the modulation of G protein expression involving oxidative stress, growth factor receptor activation and associated signaling pathways in diabetes/hyperglycemia in VSMC and their potential role in the development of vascular complications observed in diabetes.

Keywords G-proteins • Hyperglycemia • Diabetes • Signalling pathway • Vascular disease • Vascular smooth muscle • Oxidative stress

13.1 Introduction

Chronic hyperglycemia is an important contributing factor in impaired contractility and cell proliferation leading to various cardiovascular complications associated with diabetes [1] and [2]. However, the precise mechanism(s) responsible for hyperglycemia-induced vascular dysfunction remains poorly characterized. Guanine nucleotide regulatory proteins (G proteins) play an important role in the regulation of vascular tone, and aberrations in these mechanisms may contribute to vascular complications in hyperglycemia/diabetes.

Guanine nucleotide regulatory proteins (G-proteins) are a family of guanosine triphosphate (GTP) binding proteins that play a key regulatory role as transducers in a variety of signal transduction system. These include adenylyl cyclase/cAMP system [3] the receptor-mediated activation of phospholipase C and A2 [4, 5] and a number of hormone and neurotransmitter-regulated ionic cannels [6, 7] G proteins are heterotrimeric proteins composed of three distinct subunits; α (alpha), β (beta) and γ (gamma) subunits [8]. The α-subunits bind and hydrolyse GTP and confers specificity in receptor and effector interactions [8]. The GDP bound form of α binds tightly to βγ and is inactive, whereas the GTP bound form of α dissociates from βγ and serves as a regulator of effector proteins. All α-subunits possess intrinsic GTPase activity and hydrolyse the terminal phosphate of bound GTP to yield bound GDP and free inorganic phosphate (Pi). Upon hormone binding and receptor activation, the receptor interacts with the heterotrimeric protein to promote a conformational change and dissociation of bound GDP from the guanine nucleotide binding site. GDP is released and replaced by GTP. Binding of GTP to α-subunit induces a conformational change and promotes the dissociation of hormone receptor complex (HR) and the holo G protein into α and βγ. Both α-GDP and βγ subunits can interact with effectors. This activation cycle is terminated by intrinsic GTPase activity of α-subunit. The GDP-bound form of α -subunit has high affinity for βγ and then

reassociates with the βγ dimmer to form the heterotrimer in the basal resting state. The family of G-protein α-subunits can be subclassified according to functional or structural relationship. More than 20 mammalian Gα gene products and several alternatively spliced isoforms have been identified. These can be divided into four major subfamilies according to amino acid homology and are represented by Gsα, Giα,Gqα/α11 and α 12/α13. The G proteins Gs and Giα are implicated in the regulation of adenylyl cyclase/cAMP signal transduction system whereas Gqα, G11α are implicated in the regulation of phosphatidylinositide signaling.

The hormone-sensitive adenylyl cyclase system is composed of three components: the receptor, the catalytic subunit, and G-proteins – stimulatory (Gs) and inhibitory (Gi). Molecular cloning has revealed four different forms of Gsα having molecular weights of 45, 45, 52 kD resulting from the different splicing of one gene [9–11]. Gsα is positively coupled to adenylyl cyclase and mediates the stimulatory responses of hormones on adenylyl cyclase [12, 13]. The Gs-mediated activation of adenylyl cyclase results in the increase formation of cAMP. cAMP activates cAMP-dependent protein kinase A that induces the phosphorylation of contractile filaments, sarcolemmal and sarcoplasmic proteins, and regulates intracellular calcium homeostasis [14]. In addition, Gsα was also shown to open the Ca^{2+} channels directly by cAMP-independent mechanism [15]. In contrast, Giα protein is associated with adenylyl cyclase inhibition [12, 13]. Three distinct forms of Giα, namely, Giα-1, Giα-2, and Giα-3 have been cloned and encoded by three distinct genes [16–18]. All three forms of Giα; Giα 1–3 have been shown to be implicated in adenylyl cyclase inhibition [19] and activation of atrial Ach-K^+ channels [20]. Both the Gα and Gβγ dimer mediate G-protein signaling. Five different β subunits of 35–36 kDa and 12 γ subunits of 8–10 kDa have been identified by molecular cloning. The βγdimer is tightly associated with GDP bound chain and facilitate interaction of G-protein with a receptor molecule. The effectors regulated by Gβγ include K^+ channels, phospholipase C-β, and adenylyl cyclase [21–23]. Like α-subunit the γ-subunit is subject to a cascade of posttranscriptional modification including isoprenylation and myristoylation that contributes to βγ membrane association and the interaction of the subunits [24].

G-protein α-subunits also possess specific residues that can be covalently modified by bacterial toxins. Cholera toxin catalyzes the transfer of ADP-ribose moiety of NAD to a specific arginine residue in certain α-subunits, whereas pertussis toxin ADP-ribosylates those α-subunits that contain a specific cysteine residue near to carboxy terminus. Modification of α-subunit by cholera toxin persistently activates these protein by inhibiting their GTPase activity, whereas pertussis toxin inactivesGiα protein and thereby results in the uncoupling of receptor from the effector. G protein α-subunits are regulated by covalent modifications by fatty acids myristate and palmitate. These lipid modifications serve to anchor the subunits to the membrane and increase the interaction with other protein and also increase the affinity of α subunit for βγ. In this regard, the myristoylation of Giα is required for adenylyl cyclase inhibition in cell free assay [25].

13.2 G Proteins and Adenylyl Cyclase Signaling in Diabetes

Several abnormalities in G-proteins expression and adenylyl cyclase activity has been shown in various pathophysiological conditions including diabetes [26, 27]. The decreased expression of Giα proteins has been reported in hepatocytes from human diabetics and STZ-diabetic rats [26, 27], whereas an increase in the levels and functions of Giα was shown in diabetic adipocytes from a genetic model of diabetes [28] Livingstone et al. [29] have shown a decreased expression of Giα proteins in platelets from diabetic subjects as compared to non-diabetic subjects. In addition, diabetic retina has been shown to exhibit decreased levels and functions of Giα [30]. Hattori et al. [31] have also reported a similar decrease in Gi protein in aorta from long-term diabetic rats, however, these investigators did not examine adenylyl cyclase Gi-protein signaling in their studies. However, Weber and McLeod [32]were unable to observe any changes in the levels of Giα proteins in aorta or caudal artery from 12 to 14 week-STZ-diabetic rats as compared to control rats. Further support and involvement of Giα-2 protein in pathogenesis of diabetes has been provided by the studies showing that the overexpression of constitutively activated Giα-2 ameliorates STZ-induced diabetes in rats [33]. In addition, a complete knockout of the Giα-2 gene that has been reported to produce a metabolic state resembling type II diabetes suggests the relationship between the decreased levels of Giα protein and diabetes [34]. However, Hashim et al. [35] showed that the aorta from STZ-diabetic rats exhibits decreased expression of Giα-2 and Giα-3 but not of Gsα proteins. An unaltered expression of Gsα in hearts from STZ-induced diabetic rat have also been reported [35, 36]. A relationship between the development of diabetes and Giα protein expression was also demonstrated [35]. The rats treated with STZ showed enhanced blood glucose levels within 2 days after injection with a concurrent decrease in the levels of Giα proteins, suggesting that the decrease in the levels of Giα proteins is associated with the development of diabetes. Subsequent increase in the levels of blood glucose through day 5 resulted in further decrease in the levels of Giα proteins suggesting a close relationship between decreased levels of Giα proteins and severity of diabetes [35]. The decreased levels of Giα proteins were reflected in decreased Gi functions whereasGsα–mediated stimulatory effects of hormones on adenylyl cyclase were augmented in STZ-aorta as compared to control aorta resulting in an enhanced levels of cAMP, whereas the basal cAMP levels were reduced in diabetic aorta [35].

13.3 G Proteins and Adenylyl Cyclase Signaling in Hyperglycemia

Furthermore, aorta as well as A10 VSMC exposed to high glucose (26 mM) that simulate diabetic state also exhibited decreased levels of Giα-2 and Giα-3 proteins, whereas the levels of Gsα were not altered [37]. A correlation between the levels of

glucose (in vivo and in vitro) and decreased expression of Giα proteins was also reported and suggests that hyperglycemia may be a contributing factor in diabetes-induced decreased expression of Giα proteins [37].In addition, aortic VSMC from STZ-diabetic rats alsoshowed decreased expression of Giα proteins [38], suggesting that aortic VSMC cultured from STZ-diabetic rats retained the diabetic phenotype.

Hyperglycemia was also shown to stimulate adenylyl cyclase activity in bovine aortic endothelial cells which causes an inhibition of glucose-6-phosphate dehydrogenase and thereby results in decreased levels of NADPH that may be responsible for hyperglycemia-induced apoptosis [39]. In addition, an increased stimulation of cAMP levels by OP-1206, alpha CD, an analog of prostaglandin E1 (PGE1), was reported in sciatic nerve from STZ-diabetic rats that were shown to increase Na^+/K^+ ATPase activity [40]. In support of these studies, Hashim et al. [37]reported an increased stimulation of adenylyl cyclase activity by isoproterenol and glucagon in A10 vascular smooth muscle cells exposed to high glucose (26 mM) as compared to the cells exposed to normal glucose (5.5 mM). These enhanced stimulations were attributed to the decreased expression of Giα-2 and Giα-3 levels and not to increased levels of Gsα proteins, because the levels of Gsα proteins were not altered in hyperglycemic cells. Taken together, it may be suggested that hyperglycemia may be a contributing factor in diabetes-induced decreased expression of Giα proteins. However, Mancusi et al. [41]were unable to show any changes in Gi protein expression in human umbilical vein endothelial cells (HUVEC) exposed to high glucose for 15 days.

High glucose-induced decreased expression of Giα proteins was also reflected in decreased Gi functions [42]because angiotensin II (Ang II), oxotremorine (Oxo) and C-$ANP_{4–23}$ (a ring deleted peptide of ANP)-mediated inhibitionswere almost completely abolished in cells exposed to high glucose [43–45]. In addition, hyperglycemia-induced down-regulation of, vascular NPR-C, AT1 and arginine-vasopressin (AVP) receptors may also be responsible for a complete attenuation of inhibitory responses on adenylyl cyclase. In this context, acute hyperglycemia induced by STZ or alloxan has been shown to decrease the levels of vascular NPR-C, AT1 and arginine-vasopressin (AVP) receptors [46, 47]. Hyperglycemia has also been shown to impair voltage gated K^+ channel current in rat small coronary VSMC [48]. Since Giα proteins are implicated in the activation of K^+ channels, it may be possible that the impairment of K^+ channel activity may be attributed to the decreased levels of Giα protein induced by high glucose. On the other hand, basal adenylyl cyclase activity was significantly decreased in A10 VSMC or aorta exposed to high glucose [37]. Since decreased cAMP levels have been shown to augment cell proliferation [49], it may be possible that the decreased basal adenylyl cyclase activity and thereby decreased cAMP levels induced by high glucose may be a contributing factor in increased cell proliferation observed under hyperglycaemic conditions and diabetes [50].

13.4 Oxidative/Nitrosative Stress and G- Protein-Adenylyl Cyclase Signaling in Diabetes and Hyperglycemia

Increased oxidative stress has been shown to be an important contributing factor in the development of micro- and macrovascular complications of diabetes, which include nephropathy, retinopathy, and neuropathy as well as endothelial and vascular smooth muscle dysfunction [51–53]. Enhanced oxidative stress induced by hyperglycemia has also been reported in cultured VSMC and different tissues from STZ-diabetic rats [38, 51–53]. In addition, the contribution of enhanced production of superoxide anion (O_2^-) in the decreased expression of Giα proteins has also been reported in aortic VSMC from STZ-diabetic rats and A10 cells exposed to high glucose [38]. Antioxidants such as α-tocopherol, NAC; scavengers of O_2^- and DPI, an inhibitor of NADPH oxidase that were shown to restore the enhanced levels of O_2^- induced by hyperglycemia also restored the hyperglycemia-induced decreased expression of Giα-2 and Giα-3 to control levels [38].These studies suggest the implication of NADPH oxidase/O_2^- in hyperglycemia-evoked decreased expression of Giα proteins. In addition, hyperglycemia-induced decreased expression of Giα proteins was also shown to be attributed to the increased levels of peroxynitrite because scavengers of peroxynitrite; uric acid and MnTBAP restored the hyperglycemia-induced decreased expression of Giα proteins to control levels [38], suggesting a role of nitrosative stress in decreased expression of Giα proteins in hyperglycemia. Further, there is an accumulating evidence that supports the hypothesis that diabetes is associated with increased nitrosative stress and increased peroxynitrite formation in several tissues both in experimental animals and humans [54]. The increased levels of nitrotyrosine, a relatively specific marker of peroxynitrite formation has also been shown in different tissues from STZ-diabetic rats and diabetic subjects [55]. For example, an increased nitrotyrosine plasma levels were shown in type 2 diabetic patients [56] and increased production of iNOs-dependent peroxynitrite was shown in platelets from diabetic individuals [57]. In addition, hyperglycemia has also been reported to induce increased nitrotyrosine formation in the artery well of monkeys [58]. Taken together, it may be possible that the levels of peroxynitrite, formed by the interaction of NO and O_2^- may contribute to hyperglycemia-induced decreased expression of Giα proteins in VSMC.

Thus, we have shown that diabetes/hyperglycemia decreased the expression of Giα proteins and associated adenylyl cyclase signaling which may be attributed to the augmented levels of Ang II that enhance the nitroxidative stress by increasing the levels of O_2^- and $ONOO^-$ (Fig. 13.1). The treatment with antioxidants reversed the hyperglycemia-induced decreased expression of Giα proteins and adenylyl cyclase signaling to control levels. In this regard, the overexpression of constitutively activated Giα-2 has also been shown to improve STZ-induced diabetes in rats [33, 51].Taken together, it may be suggested that antioxidants by augmenting the decreased levels of Giα proteins-induced by high glucose may have beneficial effects in improving the cardiovascular complications of diabetes.

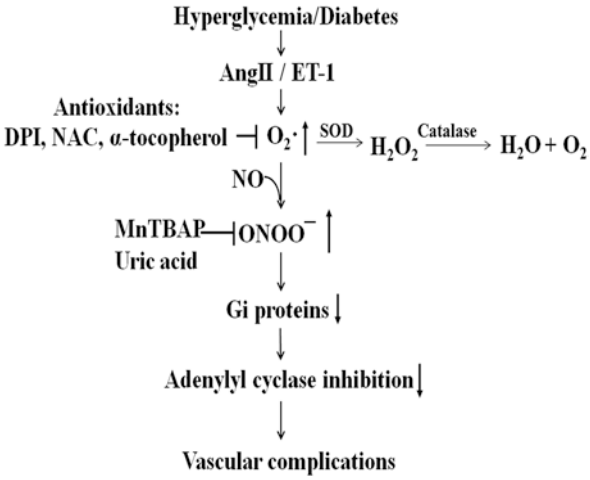

Fig. 13.1 Schematic diagram summarizing the possible mechanisms by which hyperglycemia/diabetes decreases the expression of Giα proteins and adenylyl cyclase signaling. *NO* Nitric Oxide, *ONOO-* Peroxynitrite, *SOD* Superoxide Dismutase

13.5 Gqα Proteins and Phosphatidyl Inositide Signaling in Diabetes/Hyperglycemia

The activation of Gqα by a G-protein coupled receptor (GPCR) stimulates PLCβ which hydrolyzes inositol biphosphates (PIP_2) to produce the second intracellular messengers, inositol triphosphates (IP_3) and diacylglycerol (DAG) [59, 60]. IP_3 stimulates the release of the calcium (Ca^{2+}) from the intracellular stores and DAG activates the protein kinase C (PKC). The release of intracellular calcium activates the calcium channels localized at the cell surface thus allowing the uptake of extracellular calcium inside the cell [61].

Gqα and associated signaling has been shown to play an important role in the regulation of cardiovascular functions. Alterations in the levels of $G_q\alpha$ protein and associated signaling pathways appear to contribute to the impaired cellular functions in several pathological states including diabetes, hyperglycemia, and cardiac hypertrophy [40, 62–65].A genetic ablation of $G_q\alpha$ in mice has been shown to result in a cardiac malformation and craniofacial defects [66],whereas an overstimulation of the G_q pathway in mice was shown in the development of hypertrophic cardiomyopathy [63].The $G_q\alpha$ protein and associated signaling pathway activated by several hormones such as Ang II, endothelin-1 (ET-1), and phenylephrine has also been implicated in the development and progression of cardiac hypertrophy and heart failure [67–71]. Cardiac over expression of $G_q\alpha$ in transgenic mice has also been reported to result in hypertrophy and induction of classic hypertrophy gene expression profile [72]. In addition, the transgenic over expression of a $G_q\alpha$ dominant negative mini gene that resulted in the lack of hypertrophy response to transverse aortic constriction (TAC) [62] further supports the implication of $G_q\alpha$ in hypertrophy. In addition, the $G_q\alpha$ signaling components including IP_3-Ca^{2+} and DAG-PKC have also been shown to play an important role in the development of cardiac hypertrophy in the stroke-prone spontaneously hypertensive rat (SHRSP) [73].

Furthermore, vascular G_q-coupled signaling has also been shown to contribute to the development of cardiac hypertrophy by using transgenic mice with vascular-specific $G_q$nhibitor expression [74]. We also showed the implication of G_q and MAPK/phosphatidylinositol 3-kinase signaling in VSMC hypertrophy induced by vasoactive peptides in A10 VSMC [75] In addition, Ang II-induced VSMC hypertrophy has also been shown to involve Gq signalling [76].

Diabetes-induced alterations in enhanced expression and activity of Gqα, PKC and DAG have been reported in different tissues from STZ-induced diabetic rats as well as in Bio-Breeding (BB) rats [77–81].In addition, high glucose treatment has also been reported to enhance the activity of PKC and DAG in cultured aortic endothelial and vascular smooth muscle cells (VSMC) [78, 82, 83]. The aortic VSMC from STZ diabetic rats were shown to exhibit enhanced expression of Gqα and PLCβ1 proteins [64]. Furthermore, Descorbeth and Anand-Srivastava [64] have reported that VSMC exposed to high glucose exhibit enhanced expression of Gq/11α, PLCβ-1 and PLCβ-2 proteins, the upstream signaling molecules of PI turnover and enhanced formation of IP_3 by ET-1 [64]. In addition,an increased expression of $G_q\alpha$ in sciatic nerves [77] and hearts [80, 81] from STZ-diabetic rats was also shown. However, Ceccarelli et al. [84] showed a decreased expression of $G_q\alpha$ in bovine retinal pericites exposed to 25 mmol/l of glucose [84]. In addition, an unaltered or a decreased expression of G_q/11α protein was also shown in gastric VSMCs from 10-week STZ-diabetic rats and a genetic model of non-insulin-dependent diabetes (11–12 months) WBN/Kob diabetic rats [85], respectively. The apparent discrepancies may be attributed to the difference in the cell type used and its origin.

Vasoactive peptides such as Ang II and endothelin-1 (ET-1), which can be synthesized locally in the vasculature, have been implicated in diabetes-associated vascular dysfunctions, including vascular remodeling, hypertrophy, and proliferation of VSMCs [86–91], leading to an impaired relaxation to vasodilators or an exacerbated response to vasoconstrictors. The levels of ET-1 and Ang II that are elevated in plasma from both type 1 and type 2 diabetes and also in experimental models [92–94], as well as in aortic endothelial and smooth muscle cells in the presence of high glucose [95–97], were shown to contribute to the vascular complications of diabetes. In addition, the enhanced expression of G_q/11α and PLC-β proteins in VSMC exposed to high glucose was also shown to be attributed to the enhanced levels of endogenous Ang II and ET-1 [95–97], because losartan, a selective AT_1 receptor antagonist, BQ123 as well as BQ788, ET_A and ET_B receptor antagonists, respectively, completely prevented high glucose-induced enhanced levels of G_q/11α and PLC-β protein in VSMCs and suggests that these effects may be mediated by an autocrine production of Ang II and ET-1.In this regard, the levels of various vasoactive peptides including Ang II and ET-1 which are augmented in diabetes and under hyperglycemic conditions [93–97].The underlying mechanism(s) by which AT_1 and ET_A/AT_B receptor activation by high glucose induces increased expression of Gqα and PLCβ1 proteins and vascular dysfunction involve oxidative stress because Ang II and ET-1 have increase oxidative stress by activating NADPH oxidase, an enzyme responsible for the production of superoxide anion and other reactive oxygen species [98].

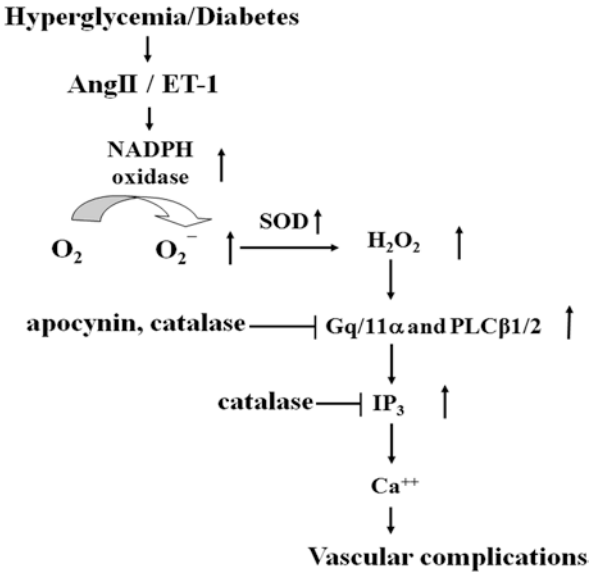

Fig. 13.2 Schematic diagram summarizing the possible mechanisms by which hyperglycemia/diabetes increases the expression of Gq/11α and associated cell signaling

The implication of enhanced oxidative stress in high glucose-induced enhanced activity/protein levels of PKC and DAG in VSMC was demonstrated by the study [99] showing that intraperitoneal injection of the antioxidant α-tocopherol into diabetic animals or the incubation of VSMCs with α-tocopherol prevented the increase in the levels of DAG and PKC due to diabetes and hyperglycemia, respectively [99]. In addition, antioxidants: apocynin, an NADPH oxidase inhibitor, and catalase, a scavenger of hydrogen peroxide but not^{111}Mn-tetrakis(benzoic acid porphyrin) (MnTBAP) and uric acid, scavengers of peroxynitrite restored the hyperglycemia/diabetes-induced enhanced levels of Gq/11α and PLCβ1/2 proteins to control levels [100] further suggest that O_{2-} and H_2O_2 but not peroxynitrite contribute to the enhanced expression of Gq/11α and PLCβ proteins in diabetes/hyperglycemia (Fig. 13.2).

13.6 Growth Factor Receptor Transactivation and Associated Cell Signaling in Gqαand PLC Protein Expression and Vascular Complications in Hyperglycemia

The role of growth factor receptor activation in VSMC hypertrophy and proliferationhas been shown [101, 102]. High glucose has also been reported to induce growth factor receptor activation/phosphorylation [103–106]. Belmadani et al. [107] showed that endothelial and VSMC from mesenteric resistance artery as well as coronary artery from db/db mice exhibited an enhanced phosphorylation of EGF-R. In addition, enhanced levels of PDGFβ-R were also shown in aortic VSMC from

STZ-diabetic rats compared with normal cells [106]. Furthermore, the implication of PDGF-R and EGF-R in high glucose-induced enhanced levels of Gq/11α and PLCβ protein as well as enhanced PLC signaling activated by ET-1 has been shown [64] and suggest that growth factor receptor activation through Gqα and PLCβ1 proteins may also contribute to VSMC hypertrophy in hyperglycemia. In addition, VSMC under hyperglycemic conditions as well as VSMC from diabetic ratsalso exhibit exaggerated cell proliferation compared to control cells [108–110]. The implication of EGF-R and PDGF-R in vascular dysfunction including remodeling, migration and proliferation of VSMC has been reported in diabetes [111–113]. This was further supported by the study showing that the inhibition of growth factor receptors, EGF-R and PDGF-R attenuated the enhanced proliferation of aortic VSMC from STZ-diabetic rats [100].

A role of MAPK and PI3K in protein synthesis and cell proliferation has been well established [42, 114–116]. Hyperglycemia has also been shown to increase the phosphorylation of ERK1/2 and AKT in human VSMC [117] and in VSMC from mesenteric resistance arteries and in A10 VSMC [100, 118] that contributes to vascular dysfunctions [118] and [117] . Campbell et al. [117]have also shown that high glucose-induced increased AKT and ERK1/2 activity was associated with VSMC chemotaxis. In addition, high glucose-induced phosphorylation of ERK1/2 was also shown to result in increased deposition of collagen in mesenteric resistance arteries [118]. In addition, MAPK, and PI3K/AKT are implicated in the hyperproliferation of VSMC from STZ-diabetic rats. The activation of MAPK and PI3K pathway by high glucose has also been shown to enhance the expression of Gqα/PLCβ proteins [119]suggesting that high glucose through Gqα/PLCβ proteins and MAPK/PI3K signaling result in VSMC hypertrophy.

The precise mechanism by which high glucose increased AKT and MAPK activity is yet not known. However, the implication of growth factor receptors in the activation of MAPK and PI3-K/AKT pathways has been shown by several studies [120–122]. Zhang et al. [123] have shown that PDGF-induced cardiac fibroblast and aortic VSMC proliferation was mediated through the activation of ERK1/2 and AKT pathways. In addition,the study showing that high glucose-induced enhanced phosphorylation of AKT and ERK1/2 was restored to control levels by growth factor receptor inhibitors suggests the implication of EGF-R and PDGF-R transactivation in high glucose-induced increased phosphorylation of AKT and ERK1/2. Taken together, it can be suggested that high glucose-induced transactivation of EGF-R and PDGF-R through the activation of AKT and ERK1/2 pathways may be responsible for high glucose-induced increased expression of Gq/11α and PLCβ proteins in VSMC. The enhanced expression of Gqα,PLCβ1 and PKCδ involving oxidative stress, growth factor receptor activation and MAP kinase signaling has been shown to contribute to VSMC hypertrophy in spontaneously hypertenive rats [101, 124, 125]. In addition, Ang II has also been reported to induce VSMC hypertrophy through Gq signaling pathway [76]. Taken together, it may be suggested that the enhanced expression of Gqα. PLCβ as well as PKC in VSMC exposed to high glucose or from diabetic rats may also contribute toVSMC hypertrophy resulting in vascular remodeling.

A role of c-Src has also been shown in high glucose-induced activation of EGF-R and PDGF-R as well as in the enhanced expression of Gq/11α and PLCβ proteins [119], and suggest that c-Src may be an upstream signaling molecule in high glucose-induced EGF-R and PDGF-R transactivation that contributes to the enhanced expression of Gq/11α and PLCβ proteins. In addition, a role of c-Src in ET-1-induced enhanced PLC signaling under hyperglycaemic conditions has also been reported [119].Furthermore, a role of Src kinases in the transactivation of EGF-R and PDGF-R has been shown [126]. In addition,Suzuki et al. [127] have shown that rat mesangial cells exposed to high glucose, exhibited an increased activity of c-Src that is associated with the increased activity of ERK5. In addition, the implication of c-Src in the proliferation of aortic VSMC from STZ-diabetic ratshas also been shown [119]. The fact that the enhanced phosphorylation of EGF-R and PDGF-R in VSMC exposed to high glucose was also attenuated by the inhibitors of c-Src suggests that c-Src through the transactivation of growth factor receptors may contribute to the enhanced cell growth in STZ-diabetic rats. In addition, the mechanism by which high glucose transactivates c-Src and thereby EGF-R and PDGF-R appears to involve oxidative stress because DPI, an antioxidant restored the high glucose-induced enhanced phosphorylation of c-Src to control levels.

Thus, we showed that high glucose transactivates EGF-R and PDGF-R through c-Src, which by activating MAPK and PI3K signaling may be responsible for the high glucose-induced enhanced expression of Gq/11α and PLCβ. The increased expression of Gq/11α and PLCβ results in enhanced production of IP_3, which by increasing the intracellular levels of Ca^{2+} may contribute to the vascular complications observed in diabetes (Fig. 13.3). In addition, the increased expression of Gqα and PLCβ may also contribute to VSMC hypertrophy and hyperproliferation, the key players of vascular remodeling resulting in vascular complications.

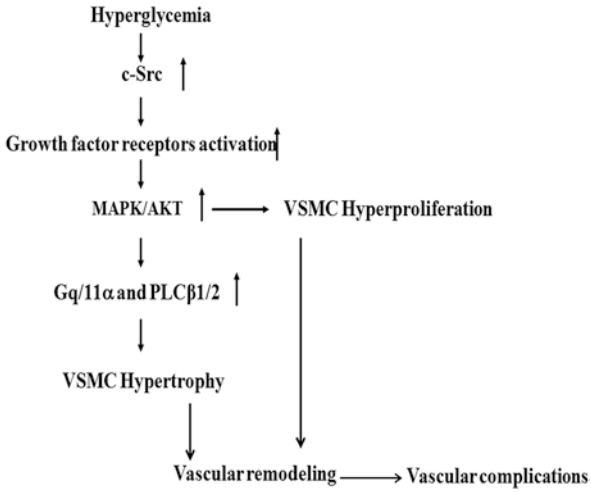

Fig. 13.3 Schematic diagram summarizing the role of C-Src and growth factor receptor activation in hyperglycemia/diabetes-induced increased expression of Gq/11α and associated – cell signaling

13.7 Conclusions

In conclusion, we have discussed the alterations in heterotrimeric G-proteins and associated functions in diabetes/hyperglycemia. We have mainly focused on Giα, Gsα and Gqα proteins which are implicated in the regulation of the adenylyl cyclase/cAMP and PLC/PI turnover, signal transduction systems respectively that play an important role in the regulation of vascular functions, including vascular tone, reactivity, VSMC proliferation and hypertrophy, the key players of vascular remodelling resulting in vascular complications. The levels of Giα-2 and Giα-3 proteins are decreased in several tissues including VSMC and aorta from STZ- induced diabetic rats as well as in VSMC exposed to high glucose., whereas the basal activity of adenylyl cyclase is attenuated resulting in the decreased levels of intracellular cAMP which may contribute to the hyperproliferation and hypertrophy of VSMC as well as to the increased vascular reactivity in diabetes and hyperglycemia (Fig. 13.4). On the other hand, the levels of Gqα/11 and PLCβ are augmented in diabetes and hyperglycemiaresulting in enhanced production of $IP_{3,}$ which by increasing the intracellular levels of Ca^{2+} may contribute to the vascular complications observed in diabetes (Fig. 13.4). In addition, the over expression of Gqα and PLCβ that has been shown to contribute to cardiac hypertrophy may also result in VSMC hypertrophy in diabetes. Taken together, it can be concluded that the alterations in the expression and functions of Giα and Gqα/PLCβ proteins in diabetes/hyperglycemia contribute to the vascular complications which may lead to cardiac failure.

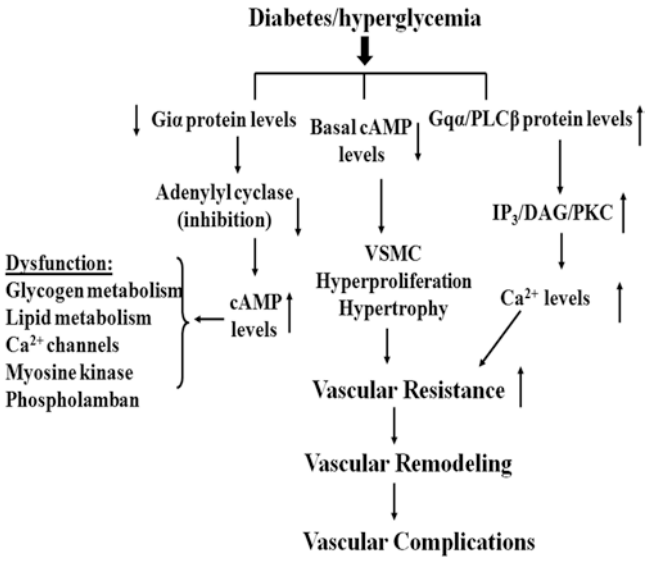

Fig. 13.4 Schematic diagram summarizing the possible mechanisms and role of altered expression of G-proteins in vascular remodeling and resultant vascular complications in diabetes/Hyper glycemia

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