Chapter 9
Role of Hyperglycemia and Redox-Induced Signaling 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. Chronic hyperglycemia appears to be an important contributing factor in this process. Various signaling pathways are implicated in diabetes/hyperglycemia-induced impaired vascular functions. Increased oxidative stress, nonenzymatic glycation, enhanced production of diacylglycerol, increased activity of protein kinase C, mitogen-activated protein kinases (MAPK), and other signaling pathways have been proposed to explain the adverse effects of hyperglycemia on vascular smooth muscle cells. Hyperglycemia-induced stimulation of the L-type Ca^{2+} channel via G-protein-coupled adenylyl cyclase/cAMP and phospholipase C/protein kinase C (PKC) pathways has also been shown. In addition, hyperglycemia has been reported to decrease the availability of nitric oxide and increase the formation of peroxynitrite which may contribute to all of the hemodynamic and physiological changes occurring in diabetes. G-protein/adenylyl cyclase signaling that plays an important role in the regulation of cardiovascular functions has also been reported to be impaired in diabetes and under hyperglycemic conditions. In this review, we have highlighted some key signaling pathways, including PKC, MAPK, and G-protein/adenylyl cyclase, which are altered in diabetes, and in response to hyperglycemia/oxidative stress, and discussed their contributions in the development of vascular complications.

Introduction

Cardiovascular disease represents a major secondary complication of diabetes and the evidence generated in recent years has suggested a strong correlation between hyperglycemia and vascular disease (Singh and Jialal 2006; Pyorala et al. 1987; Laakso 1999; Reaven et al. 2004). Although the precise mechanism by which hyperglycemia contributes to vascular disorders in diabetes remains poorly defined, a potential role of excessive generation of reactive oxygen species (ROS) in this process has been suggested (Chiasson et al. 2006) based on studies showing that

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both chronic hyperglycemia as well as spiking postprandial hyperglycemia are associated with enhanced production of ROS (Monnier et al. 2006). Further support for a role of hyperglycemia-induced ROS generation as a mediator of diabetic complications has been provided by several studies in which use of antioxidants was shown to improve cardiovascular complications of diabetes (Davi et al. 1999; Singh and Jialal 2006; Chiasson et al. 2006). Thus, there is a lot of interest to define the mechanism by which hyperglycemia contributes to ROS generation.

Multiple mechanisms for hyperglycemia-induced ROS generation have been suggested, which include increased polyol pathway, enhanced production of advanced glycation end products (AGE), and increased levels of diacylglycerol (DAG) and PKC activities (Brownlee 2001; Koya and King 1998). PKC-dependent activation of NAD(P)H oxidase has been suggested as a major source of ROS generation in response to hyperglycemia (Singh and Jialal 2006; Lee et al. 1999).

Diabetes and hyperglycemia also induce an increase in expression levels of endothelin-1 (ET-1) in several tissues (Khan and Chakrabarti 2003; Farhangkhoee et al. 2006). Similarly, hyperglycemia also increases the formation of angiotensin II (Ang II) (Koka et al. 2006; Lavrentyev et al. 2007). Both ET-1 and Ang II also enhance NAD(P)H oxidase activity and ROS generation (Lee et al. 2003). Thus, diabetes-induced ET-1 and Ang II generation and associated increase in ROS and upregulation of several key signaling systems have been implicated in the pathogenesis of aberrant vascular function. The aim of this article is to provide an overview on some of the key signal transduction pathways, most notably PKC, mitogen-activated protein kinases (MAPK), and G-protein/adenylyl cyclase systems, which are regulated by hyperglycemia and ROS.

Hyperglycemia, ROS, and PKC

PKC is a serine/threonine protein kinase comprised of at least 11 isozymic forms (Nishizuka 1995; Liu and Heckman 1998). These isozymic forms have been classified as atypical, classical, and novel. Classical PKCs (α, βI, βII, and γ) are activated by Ca^{2+}, DAG, phosphatidylserine (PS), and the tumor promoter phorbol 12-myristate 13-acetate (PMA). Novel PKCs (δ, ε, η, μ, and θ) are activated by DAG, PS, and unsaturated fatty acids, while atypical PKCs (ζ, λ, and ι) are insensitive to DAG but are activated by PS and phosphatidylinositides (reviewed in Liu and Heckman 1998; Newton and Johnson 1998; Nakanishi et al. 1993). PKCs have been implicated in a wide variety of cellular responses, including growth, differentiation, gene expression, angiogenesis, contractility, and vesicle trafficking (Nishizuka 1995).

Both PKC-βII and PKC-δ isoforms have been shown to be activated in aortic and vascular smooth muscle cells (VSMC) from diabetic rats, as well as in response to hyperglycemia (Koya and King 1998; Craven and DeRubertis 1989; Inoguchi et al. 1992; Kunisaki et al. 1994; Lee et al. 1999). Treatment of VSMCs with 22 mM glucose for 3 days increased the levels of DAG as well as PKC-βII by 50 and 110%,

respectively, compared to VSMC incubated with 5.5 mM glucose (Kunisaki et al. 1994). Under these conditions, no changes in PKC-α levels were detected (Kunisaki et al. 1994). Interestingly, α-tocopherol treatment of diabetic animals or incubation of VSMC with α-tocopherol prevented the DAG and PKC elevation due to diabetes or hyperglycemia (Kunisaki et al. 1994).

Hyperglycemia has also been found to activate total PKC activity in particulate fractions of VSMC, which was almost completely blocked by pretreatment of cells with the antioxidants probucol and D-α-tocopherol (Yasunari et al. 1999). The contribution of DAG–PKC that activates NADPH oxidase in the enhanced production of ROS has been reported in diabetic tissues (Inoguchi et al. 2003) and in cultured endothelial as well as in aortic VSMC exposed to high glucose (Inoguchi et al. 2000). In addition, expression of NADPH oxidase components was shown to be upregulated in vascular tissues and kidney from animal models of diabetes as well as in micro- and macrovascular tissues in diabetic patients and obese subjects (Inoguchi et al. 2000, 2003; Inoguchi and Nawata 2005). The increased levels of various vasoactive peptides including Ang II and endothelin in diabetes and under hyperglycemic conditions (Hargrove et al. 2000; Park et al. 2000; Sodhi et al. 2003) may contribute to the enhanced oxidative stress, because these peptides have been shown to increase oxidative stress by activating NADPH oxidase (Touyz et al. 2004). In this regard, we have recently reported that Ang II treatment of VSMC increased the production of O_2^- and the expression of Nox^4 and p^{47phox}—two subunits of NADPH oxidase (Li et al. 2007). Furthermore, AT_1 receptor blocker candesartan and ACE inhibitor quinapril have been shown to attenuate the enhanced expression of p^{47phox} in kidney from STZ-diabetic rats, implicating Ang II in diabetes-induced increased oxidative stress (Onozato et al. 2002). An increase of total cellular PKC in cultured human VSMC has also been demonstrated in response to high glucose (Williams et al. 1997). Results showing that an orally active inhibitor of PKC-β, LY33353, reversed some of the vascular abnormalities in diabetic rats have strengthened the proposed role of PKC as a mediator of diabetic vascular complications (Ishii et al. 1996).

Hyperglycemia, ROS, and MAPK

MAPK are serine/threonine protein kinases, which are activated in response to a variety of external stimuli, including growth factors, hormones, and stress. MAPK have been classified into several subfamilies: MAPK ERK 1/2 (extracellular signal-regulated kinases 1 and 2), $p38^{mapk}$, JNK/SAPK (c-Jun NH_2-terminal kinase/stress-activated protein kinase), ERK 3/4, ERK 5 (reviewed in Seger and Krebs 1995; Widmann et al. 1999). MAPK are activated by dual phosphorylation on both tyrosine and threonine residues by dual-specificity protein kinases known as MAPKK or MEK (*m*itogen *e*xtracellular signal-regulated kinase *k*inase) (Seger and Krebs 1995). The sequential upstream signaling molecules to MEK are Raf, serine/threonine kinase, and ras, a small GTP-binding protein (Widmann et al. 1999). MAPK phosphorylate downstream cytosolic and nuclear substrate/transcription

factors, such as $p90^{rsk}$, and many transcription factors, such as c-Jun, ATF-2, Elk-1, CHOP, CREB, and MEF-2 (Widmann et al. 1999; Ip and Davis 1998; Force and Bonventre 1998; Davis 1993; Denton and Tavare 1995; Tan et al. 1996; Wang and Ron 1996; Gupta et al. 1995; Zinck et al. 1995). $P90^{rsk}$ phosphorylates ribosomal proteins and participates in protein synthesis (Frodin and Gammeltoft 1999), whereas the phosphorylation of transcription factors by MAPK leads to activation of several genes involved in growth and differentiation (Widmann et al. 1999). Thus, activation of the MAPK pathway can potentially result in increased growth, gene expression, and proliferation of VSMC in response to hyperglycemia. Hyperglycemia has been shown to activate several members of the MAPK family. For example, in porcine VSMC, hyperglycemia (25 mM glucose) markedly stimulated the activation state of ERK 1/2, JNK/SAPK as well as $p38^{mapk}$ (Natarajan et al. 1999). The impact of hyperglycemia on ERK and JNK/SAPK activation was detectable within 1 hour of treatment of VSMCs whereas at least 3-hour exposure with high glucose was required to elicit any stimulatory effect on $p38^{mapk}$ activity (Natarajan et al. 1999). A similar effect of hyperglycemia on ERK 1/2 and $p38^{mapk}$ activity in rat aortic VSMC (Igarashi et al. 1999) has been reported, where high glucose caused a 3- to 4-fold increase in $p38^{mapk}$ phosphorylation, as compared to cells treated with low glucose (Igarashi et al. 1999). A general inhibitor of PKC suppressed $p38^{mapk}$ activation, suggesting an involvement of PKC in the response (Igarashi et al. 1999). More recently, an increase in the total, as well as phosphorylated forms of ERK 1/2, JNK-1, and $p38^{mapk}$ were shown to be significantly elevated in aorta isolated from STZ-diabetic rats (Igarashi et al. 2007). In these studies, VSMCs cultured from diabetic aorta exhibited a heightened phosphorylation of ERK 1/2 and $p38^{mapk}$ in response to inflammatory cytokines, such as interleukin-1β and TNF-α (Igarashi et al. 2007).

In the last few years, many reports have documented ROS-induced activation of the MAPK pathway in different cell types, including Rat-2 fibroblasts (Esposito et al. 2003), rabbit renal proximal tubular cells (Zhuang and Schnellmann 2004), Chinese hamster ovary (CHO) cells (Mehdi et al. 2005), rat cardiomyocytes and heart fibroblasts (Purdom and Chen 2005) as well as VSMCs (Blanc et al. 2003, 2004; Tabet et al. 2005). Moreover, ROS generation was shown to be critical in the activation of MAPK by Ang II (Ushio-Fukai et al. 1998; Touyz et al. 2004) and ET-1 (Daou and Srivastava 2004; Touyz et al. 2004) in VSMCs.

Several lines of evidence have indicated that an aberrant activation of MAPK is often associated with vascular remodeling in cardiovascular diseases. For example, the enhanced activation of vascular MAPK has been demonstrated in different models of vascular disease (Xu et al. 1996; Touyz et al. 2002) and a heightened activation of MAPK has been implicated in vascular remodeling, which is a hallmark of vascular disease. MAPK are also activated by ROS, such as H_2O_2 or O_2^- in a variety of cell types, including VSMCs and cardiomyocytes (Azar et al. 2006, 2007). It may thus be suggested that diabetes/hyperglycemia-induced upregulation of Ang II/ET-1 as well as an enhanced generation of ROS, and resultant activation of PKC and MAPK pathways contributes to abnormal vascular functions due to heightened growth, migration, and proliferation of VSMC (Figure 9.1).

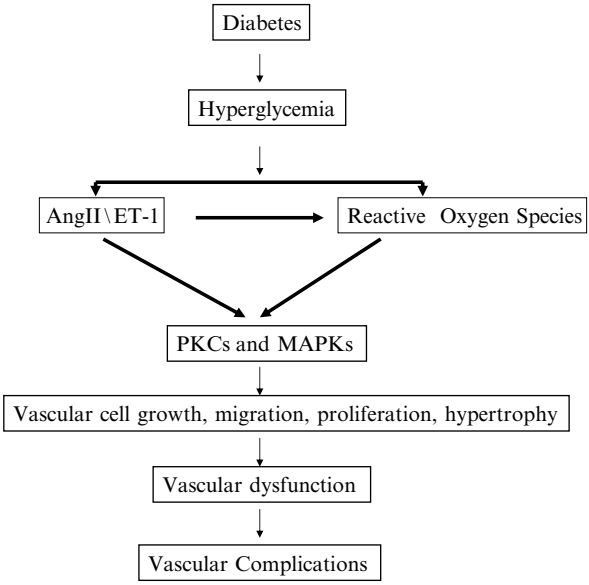

Fig. 9.1 Schematic model depicting the key events which mediate diabetes-associated vascular complications. Diabetes/hyperglycemia augments the levels of vasoactive peptides including Ang II/ET-1 that enhance the generation of reactive oxygen species (ROS). ROS-induced activation of growth-promoting signaling pathways, such as PKC and MAPK, contributes to aberrant vascular functions.

Hyperglycemia/Diabetes, ROS, and G-protein/Adenylyl Cyclase System

In addition to PKC and MAPK pathways, the adenylyl cyclase/cAMP system is also modified in diabetes and in response to hyperglycemia. The adenylyl cyclase/cAMP is one of the signal transduction systems implicated in the regulation of cardiovascular functions including arterial tone, reactivity, and cell proliferation. The hormone-sensitive adenylyl cyclase system is composed of three components: receptor, catalytic subunit, and G-proteins—stimulatory (Gs) and inhibitory (Gi)—which mediate the stimulatory and inhibitory responses of hormones on adenylyl cyclase respectively (Gilman 1984; Stryer and Bourne 1986; Spiegel 1987). G-proteins are heterotrimeric proteins composed of α, β, and γ subunits, and the specificity of G-proteins is attributed to α-subunits (Stryer and Bourne 1986). Four different forms of Gsα and three distinct forms of Giα—Giα-1, Giα-2, and Giα-3—have been identified. All three forms of Giα are implicated in adenylyl cyclase inhibition (Wong et al. 1992). Both the Gα and G$\beta\gamma$ mediate G-protein signaling. Five different β-ubunits of 35–36 kDa and seven γ-subunits of 8–10 kDa have been identified by molecular cloning (Strathmann et al. 1989; Cali et al. 1992). The effectors regulated by G$\beta\gamma$ include K^+ channels, phospholipase C-β, and adenylyl cyclase

(Strathmann et al. 1989; Tang and Gilman 1991; Wickman et al. 1994) Of the nine types of adenylyl cyclase that have been cloned and expressed (Ishikawa and Homcy 1997) only two types, types V and VI, have been identified in heart, aorta, and brain (Katsushika et al. 1992; Premont et al. 1992). Adenylyl cyclase types II and IV are activated by $G\beta\gamma$ in the presence of $Gs\alpha$, type I is inhibited by $G\beta\gamma$, and types III, V, and VI do not appear to be directly regulated by $G\beta\gamma$ (Toro et al. 1987; Taussig et al. 1993).

Diabetes, G-proteins, and Adenylyl Cyclase Signaling

Several abnormalities in G-protein expression and adenylyl cyclase activity have been shown in various pathophysiological conditions including diabetes (Lynch et al. 1989). The decreased expression of $Gi\alpha$ proteins has been reported in hepatocytes from human diabetics and STZ-diabetic rats (Bushfield et al. 1990; Caro et al. 1994), whereas an increase in the levels and functions of $Gi\alpha$ was shown in diabetic adipocytes from a genetic model of diabetes (Strassheim et al. 1991). Livingstone et al. (1991) have shown a decreased expression of $Gi\alpha$ proteins in platelets from diabetic subjects as compared to nondiabetic subjects. In addition, diabetic retina has been shown to exhibit decreased levels and functions of $Gi\alpha$ (Hadjiconstantinou et al. 1988). Hattori et al. (2000) 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 (1997) were unable to observe any changes in the levels of $Gi\alpha$ proteins in aorta or caudal artery from 12- to 14-week STZ-diabetic rats as compared to control rats. Further support and involvement of $Gi\alpha$-2 protein in the pathogenesis of diabetes has been provided by studies showing that the overexpression of constitutively activated $Gi\alpha$-2 ameliorates STZ-induced diabetes in rats (Zheng et al. 1998). In addition, a complete knockout of the $Gi\alpha$-2 gene that has been reported to produce a metabolic state resembling type II diabetes suggests the relationship between the decreased levels of $Gi\alpha$ protein and diabetes (Moxham and Malbon 1996). However, Hashim et al. (2002) have recently shown that the aorta from STZ-diabetic rats exhibited decreased expression of $Gi\alpha$-2 and $Gi\alpha$-3 but not of $Gs\alpha$ proteins. An unaltered expression of $Gs\alpha$ in hearts from STZ-induced diabetic rats has also been reported (Griffiths et al. 1990; Hashim et al. 2002). A relationship between the development of diabetes and $Gi\alpha$ protein expression was also demonstrated (Hashim et al. 2002). The rats treated with STZ showed enhanced blood glucose levels within 2 days after injection with a concurrent decrease in the levels of $Gi\alpha$ proteins, suggesting that the decrease in the levels of $Gi\alpha$ 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 $Gi\alpha$ proteins, suggesting a close relationship between decreased levels of $Gi\alpha$ proteins and severity of diabetes (Hashim et al. 2002). The decreased levels of $Gi\alpha$ proteins were reflected in decreased Gi functions, i.e., the inhibitory effect of $GTP\gamma S$ on FSK-stimulated adenylyl cyclase

activity was attenuated (Hashim et al. 2002). In addition, the inhibitory effect of Ang II, oxotremorine, and C-ANP$_{4–23}$ on adenylyl cyclase activity was completely abolished (Hashim et al. 2002). On the other hand, the Gsα-mediated stimulatory effects of hormones as well as of FSK and NaF on adenylyl cyclase were augmented in STZ-aorta as compared to control aorta resulting in enhanced levels of cAMP, whereas the basal cAMP levels were reduced in diabetic aorta (Hashim et al. 2002).

Hyperglycemia, G-proteins, and Adenylyl Cyclase Signaling

We have further shown that 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 (Hashim et al. 2004). The decreased expression of Giα proteins was concentration and time dependent. A significant decrease was observed at 20 mM glucose; below that concentration, the levels of Giα proteins were not altered. However, VSMC exposed to higher concentration of glucose (52 mM) or treated for a longer period of time (96 hours) resulted in further decrease in the levels of Giα proteins, whereas the levels of Gsα were not altered under these conditions (Hashim et al. 2004). These data indicate a correlation between the levels of glucose (*in vivo* and *in vitro*) and decreased expression of Giα proteins and suggest that hyperglycemia may be a contributing factor in diabetes-induced decreased expression of Giα proteins. In addition, aortic VSMC from STZ-diabetic rats have also been shown to exhibit decreased expression of Giα proteins (Li et al. 2008), 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 (Zhang et al. 2000). In addition, an increased stimulation of cAMP levels by OP-1206 alpha-CD, an analog of prostaglandin E_1 (PGE_1), was reported in sciatic nerve from STZ-diabetic rats that were shown to increase Na^+/K^+ ATPase activity (Yasuda et al. 1994). In support of these studies, Hashim et al. (2004) have recently 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 shown to be 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 hyperglycemia cells. Taken together, it can be suggested that hyperglycemic may be a contributing factor in diabetes-induced decreased expression of Giα proteins. However, Mancusi et al. (1996) 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 shown to be reflected in decreased Gi functions (Hashim et al. 2006). For example, Ang II, oxotremorine (Oxo), and C-ANP$_{4–23}$ (a ring-deleted peptide of ANP) which inhibit adenylyl cyclase activity through Gi proteins (Tucek et al. 2001; Anand-Srivastava et al. 1987; Anand-Srivastava 1989) inhibited the enzyme activity by about 20, 40, and 25%, respectively, in control cells. However, the exposure of these cells to high glucose eliminated the inhibitory effect of Ang II and C-ANP$_{4–23}$, whereas Oxo-mediated inhibition was only diminished by 50%. In addition, GTPγS inhibited FSK-stimulated activity (receptor-independent functions of Giα) in a concentration-dependent manner in control cells, which was almost completely abolished in cells exposed to high glucose, suggesting a correlation between decreased levels of Giα proteins. Thus, it appears that about 50–60% decrease in Giα-2 and Giα-3 proteins by high glucose may be sufficient to inhibit Gi functions and to uncouple the hormone receptors from the adenylyl cyclase system, or alternatively, some other mechanisms at the receptor level, such as receptor downregulation, 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 ANP-C, AT1, and arginine-vasopressin (AVP) receptors (Kook et al. 2002; Williams et al. 1992). Hyperglycemia has also been shown to impair voltage-gated K^+-channel current in rat small coronary VSMC (Liu et al. 2001). Since Giα proteins are implicated in the activation of K^+ channels, it may be possible that the impairment of K^+-channel activity is the result of 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 (Hashim et al. 2004). Since decreased cAMP levels have been shown to augment cell proliferation (Hayashi et al. 2000), it may be possible that the decreased basal adenylyl cyclase activity and thereby decreased cAMP levels induced by high glucose is a contributing factor in increased cell proliferation observed under hyperglycemic conditions and diabetes (Fujita et al. 2002).

Hyperglycemia, Oxidative/Nitrosative Stress, and G-protein/Adenylyl Cyclase Signaling

Hyperglycemia-induced enhanced oxidative stress has also been reported in cultured VSMC and different tissues from STZ-diabetic rats (Baynes and Thorpe 1999; Baynes 1991; Cai and Harrison 2000). In addition, the contribution of enhanced production of superoxide anion (O_2^-) in the decreased expression of Giα proteins has recently been reported in aortic VSMC from STZ-diabetic rats and A10 cells exposed to high glucose (Li et al. 2008). Antioxidants such as α-tocopherol and NAC—scavengers of O_2^-—and DPI (an inhibitor of NADPH oxidase) that restored 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 (Li et al. 2008). These studies implicate NADPH oxidase/O_2^- in

hyperglycemia-evoked decreased expression of Giα proteins. We also showed that antioxidants that restored the hyperglycemia-induced decreased expression of Giα proteins to control levels also restored to control levels the decreased Gi-mediated functions (receptor-dependent and -independent), as demonstrated by the restoration of decreased inhibition of adenylyl cyclase by Ang II, C-ANP$_{4–23}$, and oxotremorine to control levels (Li et al. 2008). In addition, GTPγS-mediated decreased inhibition of forskolin-stimulated adenylyl cyclase activity (receptor-independent functions of Giα proteins) as well as enhanced stimulation of adenylyl cyclase by GTPγS and stimulatory hormones such as isoproterenol and glucagon in hyperglycemic cells were also restored to control levels by DPI (Li et al. 2008).

Hyperglycemia-induced decreased expression of Giα proteins was shown to be attributed to the increased levels of peroxynitrite because scavengers of peroxynitrite—uric acid and MnTBAP—were able to restore the hyperglycemia-induced decreased expression of Giα proteins to control level (Li et al. 2008).The implication of peroxynitrite in NO-induced decreased expression of Giα proteins in aortic and A10 VSMC has recently been reported (Bassil and Anand-Srivastava 2006b). In addition, the treatment of VSMC with peroxynitrite has also been reported to result in decreased expression of Giα proteins (Bassil and Anand-Srivastava 2006a). There is accumulating evidence supporting the hypothesis that diabetes is associated with increased nitrosative stress and peroxynitrite formation

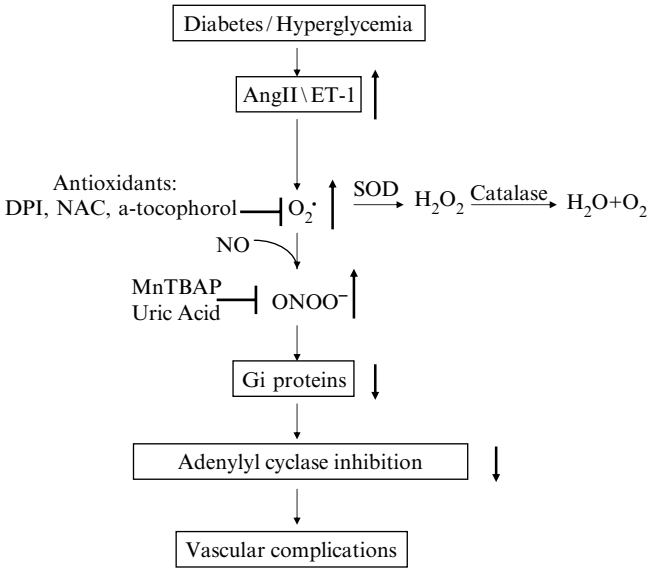

Fig. 9.2 Schematic diagram depicting the possible mechanisms by which hyperglycemia/diabetes decreases the expression of Giα proteins and adenylyl cyclase signaling. Diabetes/hyperglycemia augments the levels of vasoactive peptides including Ang II/ET-1 that enhance the oxidative stress by increasing the levels of superoxide anion (O_2^-) and peroxynitrite ($ONOO^-$). O_2^- and $ONOO^-$ decrease the levels of Gi proteins.The treatment with antioxidants and $ONOO^-$ scavengers reverses the hyperglycemia-induced decreased expression of Giα proteins and adenylyl cyclase signaling.

in several tissues both in experimental animals and in humans (Pacher et al. 2005). The increased level of nitrotyrosine, a relatively specific marker of peroxynitrite formation, has been shown in different tissues from STZ-diabetic rats and diabetic subjects (Pacher and Szabo 2006). For example, increased nitrotyrosine plasma levels were shown in type 2 diabetic patients (Cerillo et al. 2001) and iNOs-dependent peroxynitrite production was shown to be increased in platelets from diabetic individuals (Tannous et al. 1999). In addition, hyperglycemia has also been reported to induce increased nitrotyrosine formation in the artery wall of monkeys (Pennathur et al. 2001). It thus may be possible that hyperglycemia-induced enhanced levels of peroxynitrite, formed by the interaction of NO and O_2^-, may contribute to hyperglycemia-induced decreased expression of Giα proteins in VSMC. Taken together, diabetes/hyperglycemia was associated with a decreased expression of Giα proteins and associated adenylyl cyclase signaling which may be attributed to the augmented levels of vasoactive peptides such as Ang II and ET-1 that enhance the oxidative stress by increasing the levels of O_2^- and $ONOO^-$ (Figure 9.2). The ability of antioxidants to reverse the hyperglycemia-induced decreased expression of Giα proteins and adenylyl cyclase signaling to control levels suggests a key role of ROS generation in this process.

Conclusion

Diabetes is associated with alterations in the activation of several signaling events which play a key role in mediating cell growth, hypertrophy, migration, proliferation, and contractility. Many of these signaling components that include PKC, MAP kinase, G-proteins, and adenylyl cyclases are also activated in response to hyperglycemia and ROS. Dysregulation of these pathways has been linked to the cardiovascular complications associated with diabetes and could serve as potential targets in pharmacotherapy of the disease.

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