Role of oxidative stress in high glucose-induced decreased expression of G_{\alpha} proteins and adenylyl cyclase signaling in vascular smooth muscle cells

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Li Y, Descorbeth M, Anand-Srivastava MB. Role of oxidative stress in high glucose-induced decreased expression of G_{\alpha} proteins and adenylyl cyclase signaling in vascular smooth muscle cells. Am J Physiol Heart Circ Physiol 294: H2845–H2854, 2008. First published April 25, 2008; doi:10.1152/ajpheart.91422.2007.—We have recently shown that aorta from streptozotocin (STZ)-induced diabetic rats and A10 vascular smooth muscle cells (VSMCs) exposed to high glucose exhibited decreased levels of inhibitory guanine nucleotide regulatory protein (G_{i}) proteins. In the present studies, we investigated the implication of oxidative stress in the hyperglycemia/diabetes-induced decreased expression of the G_{i} protein and adenylyl cyclase signaling in VSMCs by using antioxidants. The levels of G_{i} proteins were significantly decreased in A10 VSMCs exposed to high glucose and in aortic VSMCs from STZ-diabetic rats compared with control cells and were restored to control levels by antioxidants. In addition, N
tetralis(benzoic acid porphyrin) and uric acid, scavengers of peroxynitrite, and N\n-nitro-L-arginine methyl ester, an inhibitor of nitric oxide synthase but not catalase, also restored the high glucose-induced decreased expression of G_{i} proteins to the control levels in A10 VSMCs. Furthermore, the enhanced production of superoxide anion (O^{2\cdot}) and increased activity of NADPH oxidase in these cells were also restored to control levels by diphenyleneiodonium, an inhibitor of NADPH oxidase. In addition, the diminished inhibition of adenylyl cyclase activity by inhibitory hormones and forskolin-stimulated adenylyl cyclase activity by low concentrations of GTPgammaS as well as the enhanced stimulation of adenylyl cyclase by stimulatory agonists in hyperglycemic cells were restored to control levels by antioxidant treatments. These results suggest that high glucose-induced decreased levels of G_{i} proteins and associated signaling in A10 VSMCs may be attributed to the enhanced oxidative stress due to augmented levels of peroxynitrite and not to H_{2}O_{2}.

G proteins; antioxidants; inhibitory guanine nucleotide regulatory protein

Various complications, including contractility and increased cell proliferation, are the most common complications with diabetes, and chronic hyperglycemia seems to be an important contributing factor in this process (16, 23, 33). Various signaling mechanisms such as adenylyl cyclase/cyclic (c)AMP, phosphatidyl inositol turnover, and mitogen-activated protein kinase have been shown to be implicated in the regulation of vascular tone and cell proliferation, and an aberration of these mechanisms may contribute to vascular complications observed in diabetes/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, grouped as stimulatory guanine nucleotide regulatory protein (G_{s}) and inhibitory guanine nucleotide regulatory protein (G_{i}), which mediate the stimulatory and inhibitory responses of hormones on adenylyl cyclase, respectively (20, 40). G proteins are heterotrimeric proteins composed of $\alpha$, $\beta$, and $\gamma$-subunits. Molecular cloning has revealed four different forms of G_{i} resulting from the differential splicing of one gene (12) and three distinct forms of G_{s} (G_{s}1, G_{s}2, and G_{s}3) encoded by three distinct genes (30). All three forms of G_{i} have been shown to be implicated in adenylyl cyclase inhibition (54) and the activation of atrial acetylcholine-K^{+} channels (55).

Several abnormalities in the expression of G proteins and adenylyl cyclase regulation have been demonstrated in various pathophysiological conditions, such as heart failure and hypertension (1, 3, 19). Mice deficient in G_{i}2 have been shown to exhibit a phenotype of insulin resistance (39). In addition, recent studies showing that the overexpression of G_{i}2 ameliorates streptozotocin (STZ)-diabetes further suggest the involvement of the G_{i}2 protein in the pathogenesis of diabetes (56). Diabetes-induced alterations in G protein and adenylyl cyclase activity and its responsiveness to various hormones have been demonstrated in several tissues (21, 45, 51). We have recently shown that aorta from an STZ-induced diabetic rat exhibited a decreased expression of G_{i} proteins and associated functions (26). The decrease in the expression of the G_{i} protein was dependent on the severity of diabetes. We have further shown that aorta or vascular smooth muscle cells (VSMCs) exposed to high glucose (hyperglycemic conditions) exhibited a decreased expression of G_{i} proteins and associated adenylyl cyclase signaling, whereas the levels of G_{i} were not affected (25).

The increased oxidative stress has been reported in hypertension and other cardiovascular diseases including diabetes (10, 14). The enhanced activity/levels of protein kinase C (PKC) and diacylglycerol (DAG) induced by hyperglycemia in VSMCs has been shown to be mediated by increased oxidative stress (34) because an intraperitoneal injection of antioxidant a-tocopherol to diabetic animals or the incubation of VSMCs with a-tocopherol prevented the increase in the levels of DAG and PKC due to diabetes and hyperglycemia, respectively (34). Taken together, it may be possible that hyperglycemia-induced increased oxidative stress may also be a contributing factor in decreasing the expression of G_{i} proteins in VSMCs. To examine this possibility, the present studies were undertaken to determine the effect of antioxidant treatment on the hyperglycemia-induced decreased expres-
sion of G proteins and associated adenyl cyclase signaling in A10 VSMC. This rat embryonic thoracic aorta cell line has been shown to demonstrate characteristics similar to those of VSMCs (29) and has been a useful model to study vascular cellular processes.

MATERIALS AND METHODS

Adenosine 5′-triphosphate (ATP), cAMP, isotroporotenol, forskolin (FSK), glucagon, oxotremorine, and diphenyleneiodonium (DPI) were purchased from Sigma (St. Louis, MO). Creatine kinase, myokinase, and guanosine 5′-O-(3-thiotriphosphate) (GTPγS) were purchased from Boehringer Mannheim (Montreal, Quebec, Canada). [α-32P]ATP was from Amersham (Ontario, Canada). [Des(Glu18, Ser19, Glu20, Leu21, Cly22)atrial natriuretic peptide 4–23] (C-ANP4–23) was purchased from Peninsula (Belmont, CA). AS7 and EC2 antibodies were from Dupont (Mississauga, Ontario, Canada), whereas RM/1 antibodies were purchased from Dupont (Mississauga, Ontario, Canada) and Santa Cruz.

Animal preparation. Male Sprague-Dawley rats (200 g and 6–8 wk-old) were maintained on standard rat chow and tap water ad libitum with 12:12-h light-dark cycles in a quiet environment. Diabetes was induced by an intraperitoneal injection of STZ (60 mg/kg body wt) dissolved in sodium citrate buffer (pH 4.5) as described previously (26). Age-matched control rats were injected with an equal volume of buffer solution. Blood glucose levels were monitored from day 1 to 5 after the injection using a dextrometer (Ames). STZ-injected rats with blood glucose levels in excess of 26 mM were considered to be diabetic rats (STZ) and used in the study. The blood glucose level of control rats was 5.5 mM. The rats, after 5 days of treatment, were euthanized, and the aorta were dissected out and used for cell culture. All of the protocols used in the present study were approved by the Comité de Déontologie de L’expérimentation Sur les Animaux (Canada).

Cell culture and incubation. The A10 cells line from the embryonic thoracic aorta of rats was obtained from American Type Culture Collection. VSMCs from control and diabetic aorta were cultured as described previously (26). The thoracic aorta of rats was obtained from American Type Culture Collection. VSMCs from control and diabetic aorta were cultured as described previously (25).

Fig. 1. Effect of diphenyleneiodonium (DPI) on high glucose-induced enhanced superoxide anion (O2•–) production and NADPH oxidase activity in A10 vascular smooth muscle cells (VSMCs; A10 cells). A10 cells were incubated in the presence of 5.5 mM [control (CTL)] or 26 mM glucose (hyperglycemia) for 72 h. DPI (10 μM) was added after 48 h of glucose treatment, and O2•– production and NADPH oxidase activity were determined as described in MATERIALS AND METHODS. The results are expressed as percentages of CTL, taken as 100%. Values are means ± SE of 3 separate experiments. **P < 0.01 vs. CTL (5.5 mM); ###P < 0.01; ###P < 0.001. cpm, Counts per minute.
The cells, after treatment with DPI (10 μM), were washed in oxygenated Krebs-HEPES buffer, scraped, and placed in scintillation vials containing lucigenin solution, and the emitted luminescence was measured with a liquid scintillation counter (Wallac 1409; Perkin Elmer Life Science, St. Laurent, Quebec, Canada) for 5 min. The average luminescence value was estimated as the background value subtracted, and the result was divided by the total weight of proteins in each sample.

Statistical analysis. Results are expressed as means ± SE. Comparisons between groups were made with ANOVA in conjunction with the Newman-Keuls test. Results were considered significant at a value of $P < 0.05$.

RESULTS

Superoxide anion production in VSMCs. The results shown in Fig. 1 demonstrate that $O_2^-$ production and NADPH oxidase activity were significantly augmented in hyperglycemic A10 VSMCs compared with the control cells. DPI, an antioxidant that inhibits NADPH oxidase activity, significantly restored the enhanced levels of $O_2^-$ and NADPH oxidase activity toward control levels in hyperglycemic A10 VSMCs. These results suggest that hyperglycemia increases the production of $O_2^-$, which may be attributed to increased levels of NADPH oxidase.

Effect of antioxidants on Gi protein levels. We have recently demonstrated that A10 VSMCs exposed to high glucose for 72 h decreased the levels of $G_\alpha_2$ and $G_\alpha_3$ proteins, whereas the levels of $G_\alpha$ proteins were not altered. Since high glucose and aortic VSMCs from STZ-diabetic rats exhibit enhanced production of the $O_2^-$ anion, it was of interest to examine whether hyperglycemia and diabetes-induced decreased ex-
pression of G\(\alpha\) proteins are attributed to the enhanced oxidative stress. To test this, we studied the effect of antioxidants on the levels of G\(\alpha\) proteins, which were determined by immunoblotting techniques using specific antibodies against G\(\alpha\) proteins. As shown in Fig. 2, the levels of immunodetectable G\(\alpha\)-2 (Fig. 2, left) and G\(\alpha\)-3 (Fig. 2, right) as detected by antibody AS/7 and antibody EC/2, respectively, were significantly decreased by about 30–50% in hyperglycemic cells compared with control cells as determined by densitometric scanning (Fig. 2, bottom), whereas the treatment of VSMCs with mannitol did not alter the levels of G\(\alpha\) proteins (data not shown). The prior treatment of the cells with antioxidants tocopherol (Fig. 2A), NAC (Fig. 2B), and DPI (Fig. 2C) restored the decreased expression of the G\(\alpha\)-2 and G\(\alpha\)-3 proteins to control levels. In addition, VSMCs from STZ-diabetic rats also exhibited decreased expression of G\(\alpha\)-2 (Fig. 3A) and G\(\alpha\)-3 proteins (Fig. 3B) compared with that of the control rats, and this decreased expression of G\(\alpha\) proteins was reversed to control levels by DPI and NAC.

Implication of peroxynitrate in hyperglycemic-induced decreased expression of G\(\alpha\) protein. O\(_2^\cdot\) formed by NADPH oxidase is converted to \(\text{H}_2\text{O}_2\) by superoxide dismutase (SOD), which by the action of catalase is converted to \(\text{H}_2\text{O}\). On the other hand, O\(_2^\cdot\) can also interact with nitric oxide (NO) and form peroxynitrite (ONOO\(^-\)). The availability of NO has been reported to be decreased in diabetes and under hyperglycemic conditions (11, 21, 36), which may be due to the formation of ONOO\(^-\). We have recently shown that ONOO\(^-\) decreases the expression of G\(\alpha\) proteins in VSMCs (9). Taken together, it may be possible that the high glucose-induced decreased expression of G\(\alpha\) proteins may be attributed to the increased levels of ONOO\(^-\). To investigate this possibility, the effect of MnTBAP and uric acid, the scavengers of ONOO\(^-\), on the high glucose-induced decreased expression of G\(\alpha\) proteins was examined, and the results are shown in Fig. 4. Treatment of A10 VSMCs with high glucose decreased the expression of G\(\alpha\)-2 and G\(\alpha\)-3 proteins by about 40% and 30%, respectively, which was restored to control levels by MnTBAP (Fig. 4, A and B) and uric acid (Fig. 4, C and D), whereas catalase treatment was without effect in restoring hyperglycemia-induced decreased levels of G\(\alpha\) proteins (data not shown). In addition, the treatment of hyperglycemic cells with L-NAME, which decreases the levels of NO and thereby inhibits the formation of ONOO\(^-\), also restored hyperglycemia-induced decreased levels of G\(\alpha\) proteins to control levels (Fig. 4, E and F).

Effect of antioxidants on receptor-independent function of G\(\alpha\). Since antioxidants restored the high glucose-induced decreased levels of G\(\alpha\)-2 and G\(\alpha\)-3 proteins toward control levels, it was of interest to examine whether the restoration of the decreased levels of G\(\alpha\) proteins by antioxidants is also reflected in the restoration of decreased G\(\alpha\) functions. To investigate this, the effect of DPI on receptor-independent and receptor-dependent functions was examined in hyperglycemic A10 cells. For the receptor-independent functions of G\(\alpha\), the effect of DPI was investigated on the inhibitory effect of GTP\(_S\) (10\(^{-12}\) to 10\(^{-8}\)M) on FSK-stimulated adenylyl cyclase activity in control and hyperglycemic A10 cells. The results shown in Fig. 5 indicate that the attenuated inhibition of FSK-stimulated adenylyl cyclase activity by different concentrations of GTP\(_S\) in hyperglycemic A10 cells that was attributed to the decreased levels of G\(\alpha\) proteins compared with those of untreated control cells was reversed toward control levels by DPI. However, DPI did not have any effect on the GTP\(_S\)-mediated inhibition of FSK-stimulated adenylyl cyclase activity in control cells (data not shown).

Effect of antioxidants on receptor-dependent functions. To investigate the effect of the antioxidant on the receptor-dependent functions of G\(\alpha\) proteins, the effect of DPI, \(\alpha\)-tocopherol, and NAC on ANG II, C-ANP4–23, and oxotremorine, which inhibit adenylyl cyclase through G\(\alpha\) proteins (2, 4, 5, 32), was examined. ANG II-, C-ANP4–23-, and oxotremorine-mediated inhibition of FSK-stimulated adenylyl cyclase activity in hyperglycemic A10 cells. Figure 6 shows that ANG II-, C-ANP4–23-, and oxotremorine-mediated inhibitions of adenylyl cyclase that were significantly attenuated in hyperglycemic A10 cells were restored to control levels by antioxidant treatments.

Effect of antioxidants on G\(\alpha\)-mediated stimulation of adenylyl cyclase activity. We have previously shown that the treatment of A10 VSMCs with high glucose for 72 h augmented the G\(\alpha\)-mediated functions. To examine whether DPI could also reverse the hyperglycemia-induced enhanced effects of G\(\alpha\) proteins, the effect of DPI on the GTP\(_S\)-mediated stimulation of adenylyl cyclase was investigated in hyperglycemic A10 cells, and the results are shown in Fig. 7. GTP\(_S\) stimulated adenylyl cyclase activity in a concentration-dependent manner in control and hyperglycemic A10 VSMCs; however, as reported earlier (25), the extent of the stimulation was...
significantly increased in hyperglycemic VSMCs compared with control cells. For example, GTP\textsubscript{S} augmented the adenylyl cyclase activity by sixfold in control cells compared with 3.5-fold in hyperglycemic A10 cells, and the treatment of hyperglycemic cells with 10\textmu M DPI restored the enhanced stimulation of adenylyl cyclase toward control levels; however, DPI did not have any effect on the GTP\textsubscript{S}-mediated stimulation of adenylyl cyclase in control cells (data not shown).

Effect of antioxidants on hormonal stimulation of adenylyl cyclase activity. To investigate whether the antioxidant could also modulate the high glucose-induced increased stimulation of adenylyl cyclase by stimulatory hormones, the effect of DPI on both isoproterenol- and glucagon-stimulated adenylyl cyclase activity and was examined in hyperglycemic A10 cells. As shown in Fig. 8A, both isoproterenol and glucagon stimulated adenylyl cyclase activity to various degrees in A10 VSMCs; however, as reported earlier (25), the extent of the stimulation was significantly augmented by about 60% and 40%, respectively, in hyperglycemic cells compared with control cells, and this increase was restored to control levels by DPI treatment. In addition, the enhanced stimulation of adenylyl cyclase by sodium fluoride (NaF; \(10^{-3}\)M) and FSK (\(10^{-6}\)M), which stimulate the enzyme activity by a receptor-independent mechanism in high glucose-treated cells, was also restored to control levels by DPI treatment (Fig. 8B).

DISCUSSION

We have previously reported that aorta from 5-day STZ-diabetic rats exhibited decreased expression of \(G_i\)-2 and \(G_i\)-3 proteins and associated functions (26). A significant decrease in \(G_\alpha\) proteins was observed on day 3 of the STZ injection, when the blood glucose level was increased to 20
M, and below that concentration of blood glucose, no significant decrease in Gα proteins was detected. We have further shown that aorta as well as A10 VSMCs exposed to high glucose (26 mM) that simulate the diabetic state also exhibited decreased levels of Gα-2 and Gα-3 proteins, whereas the levels of Gα were not altered (25). The decreased expression of Gα proteins was concentration and time dependent. A significant decrease was observed at 20 mM glucose, and below that concentration, the levels of Gα proteins were not altered. These data indicate a correlation between the levels of glucose (in vivo and in vitro) and decreased expression of Gα proteins and suggest that hyperglycemia may be a contributing factor in the diabetes-induced decreased expression of Gα proteins.

In the present studies, we report that aortic VSMCs from STZ-diabetic rats, like diabetic aorta (25), also exhibit decreased expression of Gα proteins, suggesting that aortic VSMCs cultured from STZ-diabetic rats retained the diabetic phenotype. We also report that A10 VSMCs exposed to high glucose exhibit an enhanced activity of NADPH oxidase and augmented production of O2−, which contributes to the decreased expression of Gα proteins in aortic VSMCs from STZ-diabetic rats and A10 cells exposed to high glucose. In this regard, hyperglycemia-induced enhanced oxidative stress has also been reported earlier in cultured VSMCs, endothelial cells, and different tissues from STZ-diabetic rats (10, 14). Multiple cellular sources of O2− have been documented, which include NADH/NADPH and xanthine oxidases, the mitochondrial respiratory chain, the arachidonic acid cascades (including lipooxygenase and cyclooxygenase), and microsomal enzymes (13, 17). The contribution of mitochondrial O2− production induced by high glucose has been reported in endothelial cells (41, 48), which plays an important role in the pathogenesis of diabetes-associated endothelial dysfunctions. Whether the high glucose-induced enhanced production of O2− in A10 VSMCs is attributed to mitochondria needs to be investigated. However, Liu et al. (38) have recently shown that the high glucose-induced enhanced production of O2− in VSMCs was abolished by O2−, scavenger Tempol, or apocynin, a specific inhibitor of NADPH oxidase, and unaffected by rotenone, an inhibitor of mitochondrial respiratory chain complex 1, i.e., NADPH oxidase in the enhanced production of reactive oxygen species (ROS), has been reported in diabetic tissues (29) and in cultured endothelial as well as in aortic VSMCs exposed to high glucose (27). In addition, the expression of NADPH oxidase components was shown to be upregulated in vascular tissues and the kidney from animal models of diabetes as well as in cultured VSMCs exposed to high glucose.
as in micro- and macrovascular tissues in patients with diabetes and obese subjects (27–29). We have also reported that the treatment of A10 VMSCs with high glucose augmented the levels of p47<sub>phox</sub> and p22<sub>phox</sub> proteins, the subunits of NADPH oxidase (18). Taken together, it may be suggested that ADP stimulation of VSMCs increased the production of O<sub>2</sub><sup>-</sup> <sup>•</sup> in the hyperglycemia-evoked decreased expression of G<sub>α</sub> proteins. However, we have earlier shown that enhanced oxidative stress contributes to the enhanced expression of G<sub>α</sub> proteins in VSMCs from spontaneously hypertensive rats (35) and in A10 VMSCs exposed to ANG II (37). Therefore, to clarify these discrepancies, we investigated the contribution of different ROS and reactive nitrogen species in the hyperglycemia-induced decreased expression of G<sub>α</sub> proteins. Hyperglycemia, through the activation of NF-κB, has been shown to augment the expression of inducible NOS (iNOS), which increases the generation of NO (50). O<sub>2</sub><sup>-</sup> formed by NADPH oxidase activation is converted to H<sub>2</sub>O<sub>2</sub> by SOD and also reacts with NO to form a potent cytotoxin ONOO<sup>-</sup>, which may be responsible for the decreased expression of G<sub>α</sub> proteins in hyperglycemia.

We showed that the hyperglycemia-induced decreased expression of G<sub>α</sub> protein may be attributed to the increased levels of ONOO<sup>-</sup> because scavengers of ONOO<sup>-</sup>, uric acid and MnTBAP, as well as l-NAME, which inhibits the production of NO and thereby the formation of ONOO<sup>-</sup>, were able to restore the hyperglycemia-induced decreased expression of G<sub>α</sub> proteins to control levels. The implication of ONOO<sup>-</sup> in the NO-induced decreased expression of G<sub>α</sub> proteins in aortic and A10 VMSCs has recently been shown (7). In addition, we have recently shown that the treatment of VSMCs with oxidase/O<sub>2</sub><sup>-</sup> in the hyperglycemia-evoked decreased expression of G<sub>α</sub> proteins.
ONOO\(^{-}\) increased the levels of cGMP and decreased the expression of Go\(\alpha\) proteins (9). cGMP has also been shown to decrease the levels of Go\(\alpha\) proteins in VSMCs (8); however, the ONOO\(^{-}\)-induced decreased expression of Go\(\alpha\) proteins was not mediated through cGMP because 1H[1,2,4]oxadiazole[4,3-a]quinoxaline-1-one, an inhibitor of soluble guanylyl cyclase, was unable to restore the decrease expression of Go\(\alpha\) proteins to the control level (9).

There is accumulating evidence that supports the hypothesis that diabetes is associated with increased nitrosative stress and ONOO\(^{-}\) formation in several tissues both in experimental animals and humans (44). ONOO\(^{-}\) attacks various biomolecules in the vascular endothelium, vascular smooth muscle, and myocardium, leading to cardiovascular dysfunction (44). ONOO\(^{-}\) has also been reported to damage DNA and thereby triggers the activation of Poly(ADP-ribose)polymerase-1 (PARP-1), a nuclear enzyme (43). The activation of PARP-1 depletes the intracellular concentration of its substrate NAD\(^{+}\) by inhibiting the rate of glycolysis, electron transport, and ATP formation, produces the ADP-ribosylation of GAPDH, and results in cardiovascular dysfunction (43). The increased levels of nitrotyrosine, a relatively specific marker of ONOO\(^{-}\) formation, have been shown in different tissues from STZ-diabetic rats and subjects with diabetes (44). For example, increased nitrotyrosine plasma levels were shown in patients with Type 2 diabetes (15), and iNOS-dependent ONOO\(^{-}\)-production was shown to be increased in platelets from individuals with diabetes (52). In addition, hyperglycemia has also been reported to induce increased nitrotyrosine formation in the artery wall of monkeys (47). Taken together, it may be possible that hyperglycemia-induced increased levels of ONOO\(^{-}\), formed by the interaction of NO and O\(_2\), may contribute to the hyperglycemia-induced decreased expression of Go\(\alpha\) proteins in VSMCs.

We also showed that antioxidants that result in the restoration of hyperglycemia-induced decreased expression of Gi\(\alpha\) proteins to control levels also restored to the 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 \(-23\), and oxotremorine to control levels. In addition, the GTP\(\gamma\)S-mediated decreased inhibition of FSK-stimulated adenylyl cyclase activity (receptor-independent functions of Go\(\alpha\) proteins) in hyperglycemic cells was also restored to control levels by DPI. Furthermore, the hyperglycemia-induced enhanced stimulation of adenylyl cyclase by GTP\(\gamma\)S and stimulatory hormones such as isoproterenol and glucagon was also restored to control levels by DPI. This may be attributed to the Go\(\alpha\) and not to G\(\alpha\) proteins because hyperglycemia was unable to alter the levels of Go\(\alpha\) proteins in these cells. In this regard, the interaction between G\(_i\) and Go\(\alpha\) proteins has been well established. This is further substantiated by our studies showing that the restoration of decreased levels of Go\(\alpha\) proteins to control levels by the antioxidant also restored the Go\(\alpha\)-mediated augmented hormonal and GTP\(\gamma\)S-induced stimulation of adenylyl cyclase to control levels.

In conclusion, we have provided the first evidence that the diabetes/hyperglycemia-induced decreased expression of Go\(\alpha\) proteins and associated adenylyl cyclase signaling may be attributed to the augmented levels of O\(_2\) and ONOO\(^{-}\) (Fig. 9). The treatment with antioxidants reversed the hyperglycemia-induced decreased expression of Go\(\alpha\) proteins and adenylyl cyclase signaling to control levels. In this regard, the overexpression of constitutively activated Go\(\alpha\)-2 has also been shown to improve STZ-induced diabetes in rats (56). Thus, taken together, it may be suggested that antioxidants, by augmenting the decreased levels of Go\(\alpha\) proteins induced by high glucose, may have beneficial effects in improving the cardiovascular complications of diabetes.

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REFERENCES
REDOX MODULATION OF G PROTEIN EXPRESSION IN HYPERGLYCEMIA


