Modulation of G-protein expression and adenylyl cyclase signaling by high glucose in vascular smooth muscle

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Abstract

Objective: We have recently shown a decreased expression of Giα proteins and associated functions in aorta from short term (5 days) streptozotocin-induced diabetic rats. Since hyperglycemia is one of the underlying causes of diabetes-induced cardiovascular complications, it was of interest to examine if hyperglycemia may play a direct role in down regulating the expression of Giα in vascular smooth muscle cells of diabetic subjects. For this, the effect of high glucose treatment on Giα protein expression and adenylyl cyclase signaling in intact aorta and vascular smooth muscle cells (A10 cells) was investigated.

Methods: The cells were grown in normal glucose (5.5 mM) medium and were subsequently exposed to high glucose (26 mM) or normal medium for various time periods (24–96 h). Aorta from control rats were exposed to normal and high glucose medium for 72 h. The levels of G-proteins were determined by immunoblotting using specific antibodies. Adenylyl cyclase activity stimulated or inhibited by agonists was determined to examine the functions of G-proteins.

Results: The levels of Giα-2 and Giα-3 proteins in membranes from A10 cells and aorta exposed to high glucose for 3 or 4 days were significantly decreased as compared to control cells and control aorta, respectively, whereas the levels of Gsα protein were not altered. In addition, receptor-dependent and -independent functions of Giα proteins were attenuated in hyperglycemic cells, as demonstrated by inhibition of forskolin (FSK)-stimulated adenylyl cyclase activity by low concentration of GTPγS or by angiotensin II (Ang II), oxotremorine or C-ANP4–23 (a ring deleted analog of atrial natriuretic peptide). On the other hand, the stimulatory effects of GTPγS, glucagon, isoproterenol, FSK and sodium fluoride on adenylyl cyclase were significantly augmented in hyperglycemic cells as compared to control cells, whereas basal adenylyl cyclase activity was significantly lower in hyperglycemic cells as compared to control cells.

Conclusion: These results indicate that high glucose decreased the levels and functions of Giα proteins in A10 VSMC and aorta. It may thus be suggested that decreased levels and activity of Giα proteins and adenylyl cyclase signaling induced by hyperglycemia may be one of the important mechanisms contributing to the cardiovascular complications associated with diabetes.

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1. Introduction

Vascular complications including impaired contractility and increased cell proliferation are the most common complications associated with diabetes, and chronic hyperglycemia appear to be an important contributing factor in this process [1–3]. However, the precise mechanism(s) responsible for hyperglycemia-induced vascular dysfunction remains poorly characterized. Since the adenylyl cyclase/cAMP signaling plays an important role in modulating a variety of vascular functions including cell proliferation,
vascular tone and reactivity, the aberration of this pathway may contribute to vascular complications in diabetes.

The adenyl cyclase system is composed of three components: receptor, catalytic subunit and stimulatory (Gs) and inhibitory (Gi) guanine nucleotide regulatory proteins [4,5]. The stimulation and inhibition of adenyl cyclase by hormones are mediated by two distinct G-proteins, Gs and Gi, respectively, that couple the receptor to the catalytic subunit. The G-proteins are heterotrimeric and are composed of α, β and γ subunits. Molecular cloning has revealed four different forms of Gsα resulting from the differential splicing of one gene [6] and three distinct forms of Giα: Giα-1, Giα-2 and Giα-3 encoded by three distinct genes [7]. All three forms of Giα (Gia) have been reported to be implicated in adenyl cyclase inhibition [8] and activation of atrial K⁺ channels [9].

Several abnormalities in the expression of G-proteins and adenyl cyclase regulation have been demonstrated in various pathophysiological conditions, such as heart failure and hypertension [10–13]. Mice deficient in Giα-2 have been shown to exhibit phenotype of insulin resistance [14]. In addition, recent studies showing that the overexpression of Giα-2 ameliorates STZ-diabetes further suggest the involvement of Giα-2 protein in the pathogenesis of diabetes [15]. Diabetes-induced alterations in G-protein, adenyl cyclase activity and its responsiveness to various hormones have been demonstrated in several tissues [16–18]. We have recently shown that aorta from STZ-induced diabetic rat exhibited a decreased expression of Giα proteins and associated functions [19]. The decrease in the expression of Giα protein was dependent on the severity of diabetes. However, the role of hyperglycemia in diabetes-induced changes in G-protein and adenyl cyclase signaling has not been determined. The present studies were undertaken to investigate the effect of hyperglycemia on the expression of G proteins and adenyl cyclase signaling in aorta and A10 vascular smooth muscle cells. This rat embryonal thoracic aorta cell line has been shown to demonstrate characteristics similar to those of vascular smooth muscle cells [20] and has been a useful model to study vascular cellular processes.

We have shown that aorta or VSMC under hyperglycemic conditions exhibited decreased expression of Giα proteins and associated adenyl cyclase signaling, whereas the levels of Gsα were not affected.

2. Materials and methods

Adenosine triphosphate (ATP), cyclic AMP (cAMP), isoproterenol, forskolin, glucagon and oxotremorine were purchased from Sigma (St. Louis, MO, USA). Creatine kinase, myokinase and GTPyS were purchased from Boehringer Mannheim (Montreal, Quebec, Canada). [α-32P]ATP was from Amersham (Ontario, Canada). ANP9-28, C-ANPα23 were purchased from Peninsula Laboratories (Belmont, CA, USA). AS/7 and EC/2 antibodies were from Dupont (Mississauga, Ontario, Canada), whereas RM/1 antibodies were purchased from Dupont (Mississauga, Ontario, Canada) and Santa Cruz (CA, USA).

2.1. Cell culture and incubation

The A-10 cell line from embryonic thoracic aorta of rats was obtained from American Type Culture Collection (ATCC). The cells were cultured in Dulbecco’s modified Eagle’s medium containing normal glucose (5.5 mM), 10% FBS and 1% antibiotic–antimycotic (containing penicillin, streptomycin and amphotericin B) at 37 °C in 95% air and 5% CO₂ as described previously [21]. The cells were passaged upon reaching confluence with 0.5% trypsin containing 0.2% EDTA and utilized between passages 5 and 15. The confluent cells were incubated in a medium containing 2% FBS for 24 h for growth arrest. After 24 h, the cells were exposed to high glucose (26 mM) (or otherwise as indicated) for 72 h (or otherwise as indicated) in the presence of 2% FBS. This treatment maintains the cells in quiescent state without cell death as determined by trypan blue exclusion technique. Cells growing in normal glucose were used as control. Mannitol (20.5 mM) was used as a control for osmolality. The cells were harvested using a rubber cell scraper and were homogenized in a glass/Teflon homogenizer containing 10 mM Tris–HCl buffer containing 1 mM EDTA. The homogenate was centrifuged at 1000 g for 10 min. The supernatant was discarded and the pellet was resuspended in 10 mM Tris–HCl buffer containing 1 mM EDTA and used for immunoblotting and adenyl cyclase assay.

2.2. Preparation of aorta particulate fraction

Rat aorta particulate fraction was prepared as described previously [19]. The dissected aorta were incubated in the presence of 5.5 or 26 mM glucose for 72 h at 37 °C and were frozen quickly in liquid N₂. The frozen aorta were pulverized to a fine powder with a mortar and pestle cooled in liquid N₂ and were stored at −70 °C until assayed. After homogenization in a Teflon/glass homogenizer in a buffer containing 10 mM Tris–HCl buffer containing 1 mM EDTA (pH 7.5), the homogenate was centrifuged at 16,000 × g for 10 min. The supernatant was discarded and the pellet was resuspended in the 10 mM Tris–HCl buffer containing 1 mM EDTA and used for immunoblotting and adenyl cyclase assay.

2.3. Adenyl cyclase activity determination

Adenyl cyclase activity was determined by measuring [α-32P]cAMP formation from [α-32P]ATP as described previously [10,19]. The assay medium containing 50 mM glycylglycine, pH 7.5, 0.5 mM MgATP, [α-32P]ATP (1–1.5 × 10⁶ cpm), 5 mM MgCl₂ (in excess of the ATP concentration), 100 mM NaCl, 0.5 mM cAMP, 1 mM 3-isobutyl-1-methylxanthine, 0.1 μM EGTA, 10 μM guano-
sine 5’-[γ-thio]triphosphate (GTPγS) (or otherwise as indicated), and an ATP regenerating system consisting of 2 mM phosphocreatine, 0.1 mg of creatine kinase/ml and 0.1 mg of myokinase/ml in a final volume of 200 μl. Incubations were initiated by addition of the membrane preparation (30–70 μg) to the reaction mixture, which had been thermally equilibrated for 2 min at 37 °C. The reactions, conducted in triplicate for 10 min at 37 °C, were terminated by addition of 0.6 ml of 120 mM zinc acetate. cAMP was purified by co-precipitation of other nucleotides with ZnCO₃, by addition of 0.5 ml of 144 mM Na₂CO₃ and subsequent chromatography by the double-column system, as described previously [10,19].

2.4. Immunoblotting

Immunoblotting of G-protein was performed as described earlier [10,19]. After SDS/PAGE, the separated proteins were electrophoretically transferred to nitrocellulose paper (Schleicher and Schuell) with a mini transfer apparatus (Bio-Rad) at 100 V for 1 h or a semi-dry transblot apparatus (Bio-Rad) at 15 V for 45 min. After transfer, the membranes were washed twice in phosphate buffered saline (PBS) and were incubated in PBS containing 3% BSA at room temperature for 2 h. The blots were then incubated with antisera against G-proteins in PBS containing 1% BSA and 0.1% Tween-20 at room temperature for 2 h. The antigen–antibody complexes were detected by incubating the blots with goat anti-rabbit IgG (Bio-Rad) conjugated with horseradish peroxidase for 2 h at room temperature. The blots were washed three times with PBS before reaction with enhanced-chemiluminescence (ECL) Western-blotting detection reagents from Amersham. Quantitative analysis of the G-proteins was performed by densitometric scanning of the autoradiographs employing the enhanced laser densitometer (LKB Ultrascan XL) and quantified using the gel Scan XL evaluation software (version 2.1) from Pharmacia (Quebec, Canada).

2.5. Statistical analysis

Data are expressed as mean ± S.E.M and were analyzed by ANOVA in conjunction with Newman–Keuls test where applicable. Comparisons between groups (control and hyperglycemic) were made with Student’s t-test for unpaired samples. Difference between groups was considered statistically significant at P<0.05.

3. Results

3.1. Effect of high glucose on Gi-protein expression in A10 vascular smooth muscle cells and aorta

To investigate if the treatment of A10 cells with high glucose for 72 h could also mimic the effect of diabetes on Gxi protein expression, the levels of Gx proteins were determined in A10 cells by immunoblotting using specific antibodies; AS/7 antibodies against Gxi-1 and Gxi-2, EC/2 antibodies against Gxi-3 and RM/1 antibodies against Gso. As shown in Fig. 1A, AS/7 and EC/2 antibodies recognized a single protein of 40 and 41 kDa, respectively referred to as Gxi-2 (Gxi-1 is absent in aorta, [22]) and Gxi-3, respectively, from control and hyperglycemic A10 cells; however, the relative amounts of immunodetectable Gxi-2 and Gxi-3 were significantly decreased by about 30% and 50%, respectively, in hyperglycemic cells as compared to control cells as determined by densitometric scanning. On the other hand, RM/1 antibodies recognized three isoforms of Gso, Gso44, Gso47 and Gso52; however, no detectable change in the levels of Gso was observed in hyperglycemic cells. In addition, the levels of Gxi-2 and Gxi-3 proteins were also decreased by about 40% and 60%, respectively, whereas the levels of Gso protein were not altered in aorta incubated with 26 mM glucose for 72 h as shown in Fig. 1B.

Fig. 2 shows the relationship between the concentration of glucose and the expression of Gxi proteins in A10 VSMC. Glucose decreased the levels of Gxi-2 and Gxi-3 proteins in a concentration-dependent manner. The maximal decrease (70%) was observed at 52 mM glucose. However, no significant change in the levels of Gxi-2 and Gxi-3 was observed at a concentration lower than 20 mM of glucose.

In addition, the decrease in Gxi-protein expression was also dependent on the time of treatment of A10 cells with high glucose (Fig. 3). A small decrease of about 25% in the levels of Gxi-2 (A) and Gxi-3 protein (B) was observed after 12–24 h of treatment; however, after 96 h of treatment, the levels of Gxi-2 and Gxi-3 were decreased by about 70% and 80%, respectively. On the other hand, the levels of Gso were not altered even after 96 h of treatment with high glucose.

3.2. Effect of high glucose on GTPγS-mediated stimulation of adenylyl cyclase activity

Fig. 4 shows the effect of GTPγS on adenylyl cyclase activity in aorta and A10 cells treated with high glucose (26 mM). GTPγS stimulated adenylyl cyclase activity in a concentration-dependent manner in aorta exposed to 5.5 mM (control) and 26 mM glucose (hyperglycemic); however, the extent of stimulation was significantly greater in hyperglycemic aorta than control aorta (4A). At 10 μM, GTPγS stimulated adenylyl cyclase activity by about 500% in control aorta, whereas about 800% stimulation was observed in aorta exposed to high glucose. Similar results were also observed in vascular smooth muscle cells (4B). GTPγS stimulated adenylyl cyclase activity in a concentration-dependent manner in both control and hyperglycemic cells; however, the extent of stimulation was significantly higher in hyperglycemic cells. At 10 μM, GTPγS-induced stimulation of adenylyl cyclase activity in control and hyperglycemic cells was about 75% and 140%, respectively. However, the basal adenylyl cyclase activity was significantly decreased in
hyperglycemic cells and aorta as compared to control cells and control aorta (basal adenylyl cyclase activities in control and hyperglycemic cells were 67.0 ± 4.1 and 46.0 ± 3.0 pmol cAMP (mg protein·10 min)−1, respectively, and in control and hyperglycemic aorta were 125.6 ± 11.1 and 76.6 ± 6.1 pmol cAMP (mg protein·10 min)−1, respectively.

3.3. Effect of high glucose on hormonal inhibitions of adenylyl cyclase

To investigate if the decreased levels of Giα proteins induced by high glucose are also reflected in decreased Gi protein functions, the effect of high glucose on receptor-dependent and receptor-independent functions was examined. The results shown in Fig. 5A demonstrate that angiotensin II (Ang II), oxotremorine (Oxo) and C-ANP4–23 (a ring deleted peptide of ANP) which inhibit adenylyl cyclase activity through Giα proteins [14,23,24] inhibited the enzyme activity by about 20%, 40% and 25%, respectively, in control cells. However, the exposure to glucose eliminated the inhibitory effect of Ang II and C-ANP4–23, whereas Oxo-mediated inhibition was only diminished by 50%.

In addition, the effect of high glucose on receptor-independent functions of Giα was examined by studying
Fig. 2. Effect of various concentrations of glucose on the expression of G\(_i\) proteins in A10 vascular smooth muscle cells (VSMC). A10 VSMC were exposed to different concentrations of glucose (5.5 mM control (CTL) to 52 mM) for 72 h. Membranes were prepared as described in Materials and methods. The membrane proteins (50 \(\mu\)g) were resolved by SDS/PAGE and transferred to nitrocellulose, which was then immunoblotted with antibody AS/7 for Gi\(_2\)-2 (A) and EC/2 for Gi\(_2\)-3 (B) and detected by using ECL Western blotting technique as described in Materials and methods. The detection of different proteins was performed by using the chemiluminescence (ECL) Western blotting detection reagents from Amersham. The immunoblots are representative of three separate experiments (upper panel). Quantification of G proteins was performed by densitometric scanning using an enhanced laser densitometer (LKB) (lower panel). The results are expressed as a percentage of control taken as 100%. Values are means \(\pm\) SEM of three separate experiments. *\(P<0.05\), **\(P<0.01\), ***\(P<0.001\).

Fig. 3. Quantification of G protein levels in A10 vascular smooth muscle cells (VSMC) exposed to 5.5 mM (control) or 26 mM glucose (hyperglycaemic) for different time periods. A10 VSMC were incubated in the presence of 5.5 mM (control, C) or 26 mM glucose (hyperglycaemic) for different time periods (2 to 96 h). Membranes were prepared as described in Materials and methods. The membrane proteins (50 \(\mu\)g) were resolved by SDS/PAGE and transferred to nitrocellulose, which was then immunoblotted with antibody RM/1 for Gs\(_\alpha\), AS/7 for Gi\(_2\)-2 and EC/2 for Gi\(_2\)-3 and detected by using ECL Western blotting technique as described in Materials and methods. The detection of different proteins was performed by using the chemiluminescence (ECL) Western blotting detection reagents from Amersham. The immunoblots are representative of three separate experiments (left panel). Quantification of G proteins was performed by densitometric scanning using an enhanced laser densitometer (LKB) (right panel). The results are expressed as a percentage of control taken as 100%. Values are means \(\pm\) SEM of three separate experiments. *\(P<0.05\), **\(P<0.01\).
the effect of low concentrations of GTPγS on forskolin (FSK)-stimulated adenylyl cyclase activity. As shown in Fig. 5B, GTPγS inhibited FSK-stimulated activity in a concentration-dependent manner in control cells, which was almost completely attenuated in cells exposed to high glucose suggesting a correlation between decreased levels and decreased functions of Giα proteins.

### 3.4. Effect of high glucose on hormonal stimulations of adenylyl cyclase

The interaction of Gsα and Giα proteins has also been well established [25]. Since high glucose did not alter the levels of Gsα proteins in the present studies, it was of interest to investigate if decreased levels of Giα proteins...
induced by high glucose could augment Gsα-mediated stimulation of adenylyl cyclase; the results shown in Fig. 6 demonstrate that isoproterenol and glucagon stimulated adenylyl cyclase activity in control and high glucose-treated cells; however, the extent of stimulation was significantly augmented in cells exposed to high glucose. For example, isoproterenol and glucagon stimulated adenylyl cyclase activity by about 130% and 110%, respectively, in control and by about 180% and 140%, respectively, in cells exposed to high glucose.

In addition, FSK and NaF that stimulate adenylyl cyclase activity by receptor-independent mechanism also stimulated enzyme activity to various degrees in both control and cells exposed to high glucose; however, the extent of stimulation was significantly higher by about 60% and 50%, respectively, in cells exposed to high glucose.

4. Discussion

We have recently shown that aorta from short-term STZ-diabetic rats exhibited a decreased expression of Gisα proteins, which was reflected in decreased Gi functions. In the present studies, we demonstrate for the first time that high glucose may be responsible for the observed decreases in the levels of Gisα-2 and Gisα-3 proteins. These results are consistent with our earlier studies showing a correlation between the levels of blood glucose and decreased levels of Gisα proteins in aorta from STZ-diabetic rats and suggest that hyperglycemia may be a contributing factor in diabetes-induced decreased expression of Gisα proteins. However, Mancusi et al. [26] were unable to show any changes in Gi protein expression in human umbilical vein endothelial cells (HUVEC) exposed to high glucose for 15 days. The apparent discrepancies may be attributed to the difference in the cell type (A10 VSMC vs. HUVEC) or to the time of exposure (3 days vs. 15 days). Our results are in accordance with the studies of other investigators who have reported a decreased expression of Gisα in various tissues from STZ-diabetic rats [27–30]. Further support and involvement of Gisα-2 protein in the pathogenesis of diabetes has been provided by the studies showing that the overexpression of constitutively activated Gisα-2 ameliorates STZ-induced diabetes in rats [15]. In addition, a complete knockout of the Gisα-2 gene that has been reported to produce a metabolic state resembling type II diabetes suggests the relationship between the decreased levels of Gisα protein and diabetes [14]. However, an increased or unaltered expression of Gisα proteins was shown in adipocytes from a genetic model of diabetes or in aorta or caudal artery from 12- to 14-week STZ-diabetic rats [31,32], respectively. Our results showing that treatment of VSMC with high glucose for 72 h or for longer period of time did not alter the levels of Gisα proteins are consistent with the other studies performed with aorta and heart from STZ-induced diabetic rats [19,33]. However, Phan et al. [34] have reported a decreased ADP-ribosylation of Gisα in pancreatic β cells. The apparent discrepancies may be attributed to the differences in the cell type (A-10 VSMC vs. β-cells) or to the method of detection (Western blotting vs. ADP-ribosylation).

The mechanism by which glucose decreased the expression of Gisα proteins is not known; however, it may be possible that decreased cAMP levels induced by high glucose may be responsible for the observed decreases in Gisα protein expression in A10 VSMC. In this regard, N6-phenylisopropyladenosine and C-ANP4–23 that inhibit adenylyl cyclase and cAMP levels have been reported to decrease the levels of Gisα proteins in adipocytes and A10 VSMC, respectively [35,36]. On the other hand, the enhanced levels of cAMP induced by isopropanaline have been shown to augment the levels of Gisα proteins [37]. The decreased expression of Gisα proteins induced by high glucose was also reflected in the decreased functions of Gisα proteins as demonstrated by attenuation of GTPγS-mediated inhibition of FSK-stimulated adenylyl cyclase activity (receptor-independent functions) and Ang II, OXO and C-ANP4–23-mediated inhibition of adenylyl cyclase activity (receptor-independent function) in A10 VSMC.
activity (receptor-dependent functions). Our results are in agreement with previous studies performed in aorta and other tissues from STZ-induced diabetic rats [19,26,27,30,32]. 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 adenylyl cyclase system, or alternatively, some other mechanisms at the receptor level, such as receptor down-regulation, 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 [38,39]. Taken together, it may be suggested that decreased levels of Giα-2 and Giα-3 proteins and receptor downregulation in VSMC induced by high glucose may be responsible for the attenuated receptor-mediated inhibition of adenylyl cyclase by Ang II, oxytocin and C-ANP4–23. Hyperglycemia has also been shown to impair voltage gated K+ channel current in rat small coronary VSMC [40]. 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.

We have recently shown that STZ-induced diabetic aorta exhibited decreased basal adenylyl cyclase activity. In the present study we demonstrate that the exposure of aorta or A10 VSMC to high glucose also attenuated the basal adenylyl cyclase activity. The decreased basal activity was not attributed to the decreased expression of Gi because the basal activity is in the native state and is not under the influence of G-proteins. However, it may be possible that hyperglycemia decreases the expression of catalytic component of adenylyl cyclase and thereby results in the reduction of basal enzyme activity. Our results are in agreement with other studies showing a similar diabetes-induced reduction in basal adenylyl cyclase activity in various tissues [16,18,19,41]. Since decreased cAMP levels have been shown to augment cell proliferation [42], 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 hyperglycemic conditions and diabetes [43]. In addition, the augmented sensitivity of adenylyl cyclase to GTPγS stimulation in aorta and VSMC exposed to high glucose may also be attributed to the decreased basal adenylyl cyclase activity, and decreased levels of Giα proteins and not to the increased levels of Gsα, because the levels of Gsα were not augmented by high glucose.

The enhanced stimulation of adenylyl cyclase by isoproterenol and glucagon under hyperglycemic conditions may be attributed to decreased levels of Giα proteins, upregulation of hormone receptors or increased levels of Gsα proteins or to the impaired catalytic subunit. However, most of the studies performed on β-adrenergic receptor binding from STZ-diabetic rats showed a downregulation and not upregulation of receptors [44,45]. Furthermore, since no alterations in the level of Gsα were observed in response to hyperglycemia, it may be suggested that decreased levels of Giα proteins and decreased basal GTP-sensitive adenylyl cyclase activity due to impaired catalytic subunit by high glucose may be responsible for augmented responsiveness of adenylyl cyclase to stimulatory hormones. In this regard, a relationship between decreased levels of Gi proteins and augmented stimulation of adenylyl cyclase by stimulatory hormones has been shown by previous studies [29,46–48]. Our results are consistent with the observations of other investigators showing that loss of Giα functions in STZ-diabetic rats resulted in augmentation of glucagon and isoprenaline-mediated stimulation of adenylyl cyclase activity [18,32]. In addition, hyperglycemia-induced augmented stimulation of adenylyl cyclase by FSK and NaF may be attributed to the hypersensitivity or to the increased levels of catalytic subunit of adenylyl cyclase system per se or to the decreased expression of Gα or to the increased expression of Gsα or to the alterations in all the components of adenylyl cyclase system. Since the levels of Gsα proteins were not altered by high glucose, the increased sensitivity of adenylyl cyclase to FSK or NaF stimulation under hyperglycemic conditions cannot be attributed to the Gs activity. On the other hand, based on the fact that basal adenylyl cyclase activity was decreased and not increased in A10 cells exposed to high glucose, the implication of increased levels of catalytic subunit of adenylyl cyclase in enhanced stimulation of adenylyl cyclase by FSK is ruled out. Thus, it may be suggested that the decreased levels of Giα proteins and decreased basal adenylyl cyclase activity in A10 VSMC exposed to high glucose may contribute to the augmented stimulation of adenylyl cyclase by FSK. These results are in agreement with our previous studies showing an increased stimulation of adenylyl cyclase by NaF or FSK in aorta from STZ-diabetic rats [19]. In addition, decreased levels of Giα proteins induced by pathological states [46,47] and by agents such as C-ANP4–23 [36], PT [23] or amiloride [46] have also been reported to augment the sensitivity of adenylyl cyclase to FSK or NaF stimulation.

In conclusion, we have provided the first evidence to demonstrate that A10 VSMC exposed to high glucose to simulate diabetic state decreased basal adenylyl cyclase activity, levels of Giα proteins without affecting the levels of Gsα proteins. The decreased levels of Giα proteins are reflected in decreased functions of Giα and enhanced Gs-mediated functions. It is thus suggested that the decreased levels of Giα proteins and associated adenylyl cyclase signaling as well as impaired K+ channel activity demonstrated earlier [40] may be one of the contributing factors responsible for the vascular complication of diabetes. On the other hand, the decreased basal adenylyl cyclase activity and thereby decreased cAMP levels induced by high glucose may play a role in the increased cell proliferation observed under hyperglycemic conditions and diabetes.
References


