Original Article

Adiponectin Suppression of High-Glucose–Induced Reactive Oxygen Species in Vascular Endothelial Cells

Evidence for Involvement of a cAMP Signaling Pathway

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Adiponectin is an abundant adipocyte-derived plasma protein with antiatherosclerotic effects. Vascular signal transduction by adiponectin is poorly understood and may involve 5′-AMP–activated protein kinase (AMPK), cAMP signaling, and other pathways. Hyperglycemia sharply increases the production of reactive oxygen species (ROS), which play a key role in endothelial dysfunction in diabetes. Because the recombinant globular domain of human adiponectin (gAd) reduces the generation of endothelial ROS induced by oxidized LDL, we sought to determine whether adiponectin could also suppress ROS production induced by high glucose in cultured human umbilical vein endothelial cells. Incubation in 25 mmol/l glucose for 16 h increased ROS production 3.8-fold ($P < 0.05$), using a luminol assay. Treatment with gAd for 16 h suppressed glucose-induced ROS in a dose-dependent manner up to 81% at 300 nmol/l ($P < 0.05$). The AMPK activator 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR; 1 mmol/l, 16 h) only partially decreased glucose-induced ROS by 22% ($P < 0.05$). Cell pretreatment with AMPK inhibitors, however, failed to block the effect of gAd to suppress glucose-induced ROS, suggesting that the action of gAd was independent of AMPK. Interestingly, activation of cAMP signaling by treatment with forskolin (2 μmol/l) or dibutyryl-cAMP (0.5 mmol/l) reduced glucose-induced ROS generation by 43 and 67%, respectively (both $P < 0.05$). Incubation with the cAMP-dependent protein kinase (PKA) inhibitor H-89 (1 μmol/l) fully abrogated the effect of gAd, but not that of AICAR, on ROS induced by glucose. gAd also increased cellular cAMP content by 70% in an AMPK-independent manner. Full-length adiponectin purified from a eukaryotic expression system also suppressed ROS induced by high glucose or by treatment of endothelial cells with oxidized LDL. Thus, adiponectin suppresses excess ROS production under high-glucose conditions via a cAMP/PKA-dependent pathway, an effect that has implications for vascular protection in diabetes. *Diabetes* 55: 1840–1846, 2006

Adiponectin is an abundant adipocyte-derived circulating plasma protein with insulin-sensitizing metabolic effects and vascular protective properties (1–4). Low adiponectin levels are associated with endothelial dysfunction (5–7) and a predisposition to vascular injury (8–11). In cultured endothelial cells, adiponectin has been shown to exhibit various anti-inflammatory effects, in particular those that counter the adverse cellular influences of increased oxidative stress or stimulation with cytokines such as tumor necrosis factor-α (TNF-α) (2). Adiponectin binds to the walls of catheter-injured vessels (12,13) and inhibits the expression of several adhesion molecules, including vascular cell adhesion molecule-1, E-selectin, and intracellular adhesion molecule-1 induced by the cytokine TNF-α, and it reverses the adhesion of human monocytic THP-1 cells to cultured endothelial cells (14) and enhances nitric oxide (NO) production by endothelial cells (15,16). Although the metabolic effects of adiponectin in a variety of cell types have been closely associated with activation of 5′-AMP–activated protein kinase (AMPK) (17–19), diverse pathways have been implicated in adiponectin signaling in the vasculature (2).

Hyperglycemia is a key factor in the development of vascular complications in patients with diabetes (20). Hyperglycemia sharply increases the production of reactive oxygen species (ROS), which play a key role in endothelial dysfunction in diabetes (21,22). Systemic and vascular ROS production lead to reduced endothelial NO bioactivity, increased expression of cell surface adhesion molecules, and inflammatory changes that contribute to microvascular and macrovascular damage (23–27). Although most studies have linked the salutary effects of adiponectin with obesity-linked insulin resistance and type 2 diabetes, epidemiological data has also implicated a potential role for adiponectin in coronary artery disease in type 1 diabetes (28). Recently, two studies (29,30) have also demonstrated a negative association between adiponectin and oxidative stress in human subjects, suggesting the possibility that one of the systemic effects of adiponectin is to suppress ROS generation.

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AICAR, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside; AMPK, 5′-AMP–activated protein kinase; ara-A, adenine-9-β-D-arabinofuranoside; BAEC, bovine aortic endothelial cell; fAd, full-length adiponectin protein; gAd, globular domain of human adiponectin; HUVEC, human umbilical vein endothelial cell; PKA, cAMP-dependent protein kinase; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-α.

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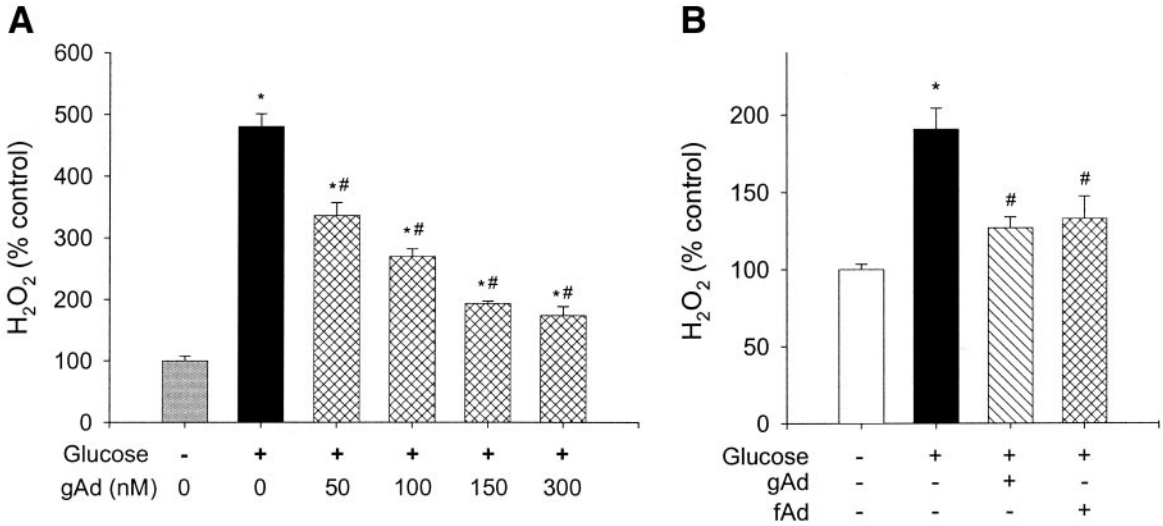

FIG. 1. Suppression of high-glucose–induced ROS production by adiponectin in endothelial cells. Endothelial cells made quiescent in basal endothelial medium were treated with adiponectin for 30 min before increasing the glucose concentration in the culture medium to 25 mmol/l. After incubation for 16 h in high-glucose medium, H_2O_2 generation was determined with the luminol assay. *A*: Response to increasing concentration of gAd in HUVECs, where the basal H_2O_2 production was 40 μmol per 3×10^5 cells per 30 min. *B*: Response to gAd (100 nmol/l) and fAd (3 μg/ml) in BAECs. Data are expressed as the percent of basal and represent the means ± SE, $n = 3$. *$P < 0.05$ vs. control; #$P < 0.05$ vs. glucose-treated cells.

Because we found that the recombinant globular domain of human adiponectin (gAd) reduces the generation of endothelial ROS induced by oxidized LDL (16), we sought in the current study to determine whether gAd could also suppress ROS production induced by high-glucose conditions in endothelial cells. We also investigated the potential signaling pathway(s) modulated by gAd that results in ROS suppression. Adiponectin is known to activate AMPK in endothelial cells (15,31,32), and recently activation of AMPK has been shown to reduce ROS derived from mitochondria in high-glucose conditions (33). However, a role for cAMP signaling has also been implicated in the effects of gAd on TNF-α–mediated inflammatory effects in endothelial cells (34). In the current work, we evaluated to contribution of both of these pathways to the suppression of glucose-induced ROS production by globular and full-length adiponectin.

RESEARCH DESIGN AND METHODS

Human umbilical vein endothelial cells (HUVECs) and endothelial basal medium-2 were obtained from Cambrex BioScience (Walkersville, MD); bovine aortic endothelial cells (BAECs) were from Cell Applications (San Diego, CA). Enhanced chemiluminescence reagents were from NEN Life Science Products (Boston, MA). The AMPK inhibitor compound C (6-[4-(2-piperidin-1-yl-ethoxy)-phenyl)]-3-pyridin-4-yl-pyyrazolo[1,5-a] pyrimidine) (35) was kindly provided by Merck Research Laboratories (Rahway, NJ). 5-amino-imidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) was from Toronto Research Chemicals (North York, ON, Canada). Human native LDL obtained from Chemicon (Temecula, CA) was oxidized and prepared as described previously (16). All other chemicals and reagents, unless otherwise noted, were obtained from Sigma (St. Louis, MO).

Recombinant globular domain and full-length adiponectin. The recombinant gAd was expressed as an NH_2-terminal $(His)_6$-tagged fusion protein in the *Escherichia coli* Origami B strain and applied to an ActiClean Etox column (Sterogene Bioseparations, Carlsbad, CA) to remove endotoxin contamination, as previously described (19). The full-length adiponectin cDNA was isolated by RT-PCR from human adipocyte mRNA and subcloned into the pCRII-TOPO vector (Invitrogen) by PCR-based subcloning. After confirming the correct cDNA sequence for human full-length adiponectin protein (fAd) lacking a signal peptide, the insert was subcloned into the vector pFLAG-CMV3 (Sigma) to acquire an NH_2-terminal FLAG epitope as well as to regain a signal peptide. The resulting vector pFLAG-fAD was transferred into 293 cells, and stable transfectants were selected by using G418. Cells were grown on Cytopore-2 microcarriers in suspension culture (BD Biosciences). The supernatant from serum-free medium was concentrated by 40% (wt/vol) ammonium sulfate precipitation and purified over an anti-FLAG M2 affinity gel (Sigma). Protein concentrations were measured, using the method of Bradford (36).

Cell culture. HUVECs or BAECs were cultured in endothelial basal medium-2 containing 10% FCS and premixed endothelial cell growth supplements following the manufacturer's instructions. Cells were routinely studied before the fourth passage and at 80–90% confluence. Before the indicated treatments, the cells were made quiescent by replacing the growth medium with human Endothelial-SFM basal growth medium (Gibco), which contains 5 mmol/l glucose and no serum or growth factor supplementation.

Cellular ROS formation in HUVECs. ROS generation was determined with a luminol assay, using an H_2O_2 standard curve (37).

Western blotting. Protein immunoblotting was performed as reported (38). Cell lysate protein (50 μg) was resolved by SDS-PAGE and transferred to polyvinylidine fluoride membranes, and immunoblotting was performed with phospho-AMPK (Thr^{172}) and AMPK protein (α1+α2 isoform) antibodies from Cell Signaling Technology (Beverly, MA). After incubation with horseradish peroxidase–conjugated secondary antibodies, proteins were visualized by enhanced chemiluminescence, according to the instructions provided by the manufacturer. Immunoblotting signals were quantitated using a Kodak ImageStation 440.

Measurement of cellular cAMP content. cAMP was measured in the HUVECs, using a direct enzyme immunoassay kit and instructions provided by the manufacturer (GE Healthcare/Amersham Biosciences).

Statistical analyses. Quantitative data are presented as the means ± SE determined from the indicated number of experiments. Statistical analysis was based on Student's t test for comparison of two groups or one-way ANOVA for multiple comparisons. $P < 0.05$ was used to determine statistical significance.

RESULTS

Adiponectin suppresses high-glucose–induced H_2O_2 production in endothelial cells. HUVECs were incubated for 16 h with 25 mmol/l glucose with and without the indicated concentrations of recombinant bacterial gAd (Fig. 1*A*). High-glucose conditions increased H_2O_2 production 3.8-fold ($P < 0.05$). Mannitol, at 25 mmol/l for 16 h as an osmotic control, was without effect, and treatment with gAd alone for 16 h did not affect the basal rate of H_2O_2 production under 5 mmol/l glucose conditions in normal culture medium (not shown). Increasing concentrations of gAd in high-glucose medium dramatically suppressed glu-

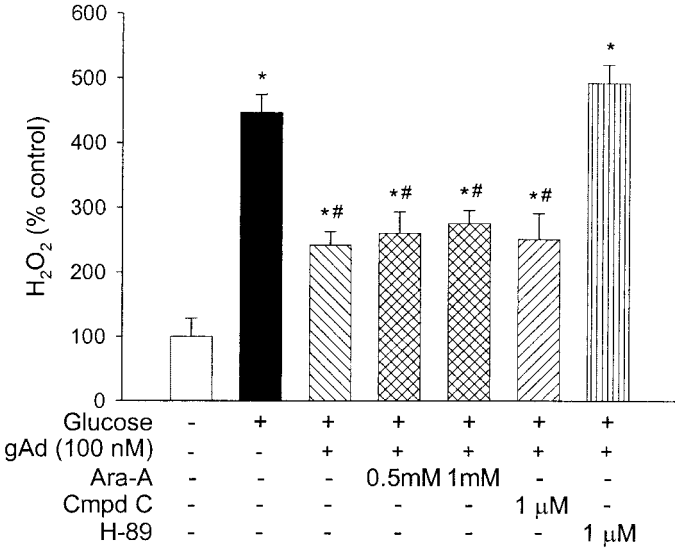

FIG. 2. Effect of inhibitors of AMPK or PKA on glucose- and/or gAd-regulated ROS generation in HUVECs. Quiescent HUVECs were stimulated without or with 100 nmol/l gAd under the following conditions: after a 15-min incubation with ara-A, compound C, or H-89, gAd was added for another 30-min incubation, after which the glucose concentration was increased to 25 mmol/l. The luminol assay for H_2O_2 was performed 16 h later. Data are expressed as the percent of basal H_2O_2 production (control) and represent the means ± SE, $n = 3$. *$P < 0.05$ vs. 5 mmol/l glucose control; #$P < 0.05$ vs. high-glucose (25 mmol/l) conditions.

cose-induced ROS in a dose-dependent manner up to 81% at 300 nmol/l ($P < 0.05$).

BAECs were incubated under identical conditions as described for HUVECs with and without recombinant gAd (100 nmol/l) or fAd (3 μg/ml) (Fig. 1*B*). High-glucose conditions increased H_2O_2 production in BAECs by 91% ($P < 0.05$). As with gAd, fAd alone did not significantly affect the basal rate of H_2O_2 production (see Fig. 7). Both gAd and fAd significantly inhibited the glucose-induced increase in H_2O_2 production in BAECs by 71 and 64%, respectively ($P < 0.01$).

Treatment of HUVECs with the NADPH oxidase inhibitors diphenyleneiodonium (5 μmol/l)) or apocynin (500 μmol/l) for 30 min before and during the 16-h period of incubation in 25 mmol/l glucose partially inhibited the increased H_2O_2 production under high-glucose conditions, by 53 and 44%, respectively (data not shown). These results suggest that ROS generation under high-glucose conditions at least partly results from cellular NADPH oxidase activity.

Effect of modulators of AMPK activity on the suppression of high-glucose–induced H_2O_2 generation by gAd in HUVECs. The AMPK pathway has been shown to be an important mediator of the cellular signaling effects of adiponectin in a variety of tissues (1). Using two inhibitors of AMPK, we next studied whether blocking AMPK signaling affected the action of gAd to reduce H_2O_2 production in high-glucose exposure (Fig, 2). Under conditions where glucose increased H_2O_2 production by 3.5-fold and 100 nmol/l gAd suppressed this effect by 59%, coincubation with the AMPK inhibitors adenine-9-β-D-arabinofuranoside (ara-A) or compound C did not significantly block the inhibitory action of gAd on H_2O_2 generation.

As assessed by phosphorylation of the AMPK Thr^{172} residue, under conditions identical to those used in Fig. 2, AMPK activation was strongly promoted by gAd (Fig. 3). Importantly, activation of AMPK by gAd was significantly blocked by ara-A and was essentially completely prevented by compound C. These results suggest that although AMPK was activated in the HUVECs by gAd treatment, AMPK did not mediate the suppression of ROS generation by gAd.

The potential role of AMPK was also further evaluated by testing whether activation of AMPK by AICAR affected the generation of H_2O_2 by high-glucose incubation. AICAR activation of AMPK was demonstrated by increased phosphorylation on Western blot (Fig. 3). In the experiment shown in Fig. 4, glucose increased H_2O_2 production by 2.6-fold, and 100 nmol/l gAd suppressed this effect by 50% ($P < 0.05$). In contrast, AICAR only reduced the glucose-induced ROS generation by 22%, indicating that activation of AMPK alone was less effective compared with gAd in suppressing ROS production in high glucose.

Effect of cAMP-dependent protein kinase inhibition on the suppression by gAd and AICAR of H_2O_2 production under high-glucose conditions. In Fig. 2, although AMPK inhibitors were without effect, the suppression of glucose-induced H_2O_2 generation by gAd was completely blocked by inhibition of cAMP-dependent protein kinase (PKA) with the inhibitor H-89. The small (22%) inhibition of glucose-induced H_2O_2 generation by activation of AMPK with AICAR was unaffected by H-89 (Fig. 4). These results suggest that although AMPK activation itself may have a small effect on suppression of glucose-induced H_2O_2 generation, it does not appear to mediate the potent suppression of ROS generation exhibited by gAd. Instead, the effect of gAd appears to be strongly mediated via PKA, which may be activated by gAd in parallel to the AMPK cascade.

Effects of increasing cAMP on glucose-induced ROS production in HUVECs. Studies complementary to those showing that blocking PKA activity abrogated the effect of gAd to suppress glucose-induced ROS generation were performed by increasing cellular cAMP action, using the mimetic dibutyryl-cAMP and forskolin, an activator of adenylyl cyclase (Fig. 5). Treatment of HUVECs with

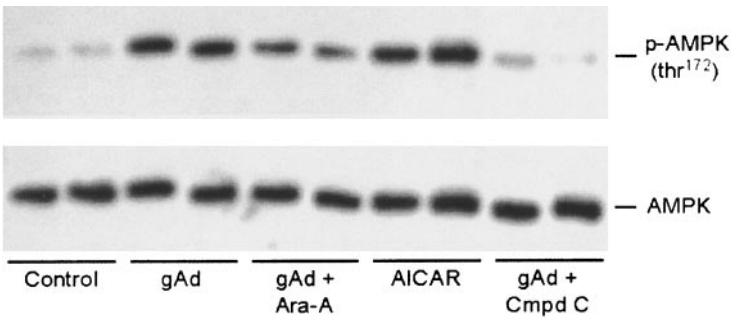

FIG. 3. Activation of AMPK by gAd and AICAR in HUVECs. Quiescent HUVECs were treated for 16 h with 100 nmol/l gAd or for 30 min with 1 mmol/l AICAR; where indicated, ara-A (1 mmol/l) or compound C (Cmpd C; 1 μmol/l) was added 15 min before the addition of gAd and maintained throughout the 16-h treatment period. Cell lysates were directly electrophoresed in SDS gels, immunoblotted with anti–phospho-AMP kinase (Thr^{172}) antibodies, and subjected to visualization with enhanced chemiluminescence, as described in RESEARCH DESIGN AND METHODS. The membranes were also stripped and reprobed with antibody reacting with the total AMPK protein level as shown.

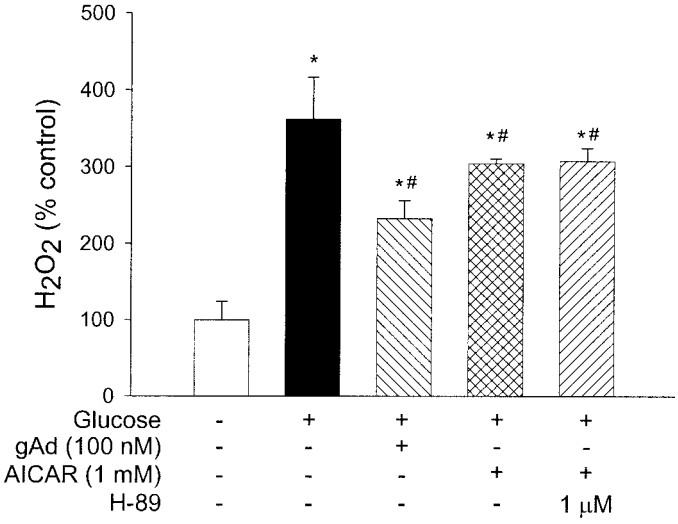

FIG. 4. Effect of AMPK activation on glucose- and/or gAd-regulated ROS generation in HUVECs. HUVECs made quiescent were treated for 15 min with H-89 or for 30 min with gAd or AICAR as indicated before increasing the glucose concentration to 25 mmol/l. The luminol assay for H_2O_2 was performed 16 h later. Data are expressed as the percent of basal H_2O_2 production (control) and represent the means ± SE, n = 3. *P < 0.05 vs. 5 mmol/l glucose control; #P < 0.05 vs. high-glucose (25 mmol/l) conditions.

dibutyryl-cAMP reduced the glucose-induced generation of H_2O_2 by 67% ($P < 0.05$). Similarly, pretreatment of cells with 2.0 μmol/l forskolin suppressed the glucose-induced generation of H_2O_2 by 43%, similar to the effect of gAd, which was 37% (both $P < 0.05$).

Effects of gAd on cAMP content in HUVECs. We next tested whether treatment of HUVECs with gAd actually increased cellular cAMP levels (Fig. 6). As controls, two expected activators of cAMP, forskolin and IBMX (3-isobutyl-1-methylxanthine), a phosphodiesterase inhibitor, increased cellular cAMP by 1.2- to 1.3-fold ($P < 0.05$). Glucose itself had no significant effect on cellular cAMP content. In the presence of high-glucose conditions, gAd significantly increased the cellular content of cAMP by 70% ($P < 0.05$). This effect of gAd was not inhibited by cellular treatment with compound C or ara-A, suggesting further that AMPK activation is not involved in the effect of gAd to increase cAMP content. The AMPK activator AICAR by itself also tended to decrease cellular cAMP content. These results showed that gAd can increase cAMP levels in endothelial cells in a manner that is independent of AMPK activation.

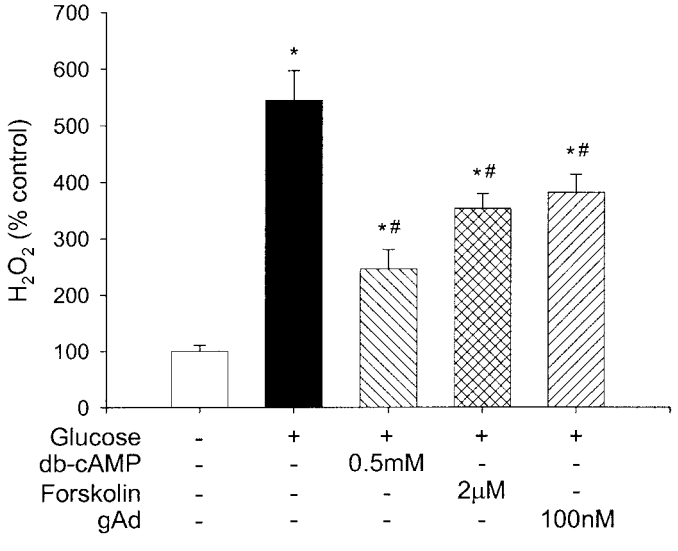

FIG. 5. Effect of increasing cAMP on glucose-induced ROS production in HUVECs. Quiescent HUVECs were treated for 15 min with dibutyryl-cAMP (db-cAMP) or forskolin or for 30 min with gAd or AICAR as indicated before increasing the glucose concentration to 25 mmol/l. The luminol assay for H_2O_2 was performed 16 h later. The data are expressed as the percent of basal H_2O_2 production (control) and represent the means ± SE, n = 3. *P < 0.05 vs. control; #P < 0.05 vs. glucose-treated cells.

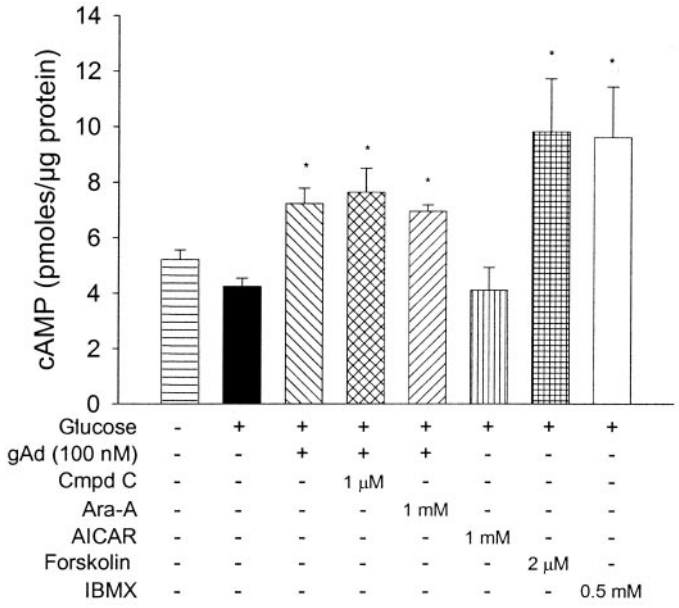

FIG. 6. Effects gAd and other agents on cAMP content in HUVECs. HUVECs made quiescent were stimulated for 15 min with the indicated agents and then for 30 min without or with gAd before increasing the glucose concentration to 25 mmol/l. The cAMP assay was performed ~16 h later, using materials in a kit from GE Healthcare/Amersham Biosciences. Data are the means ± SE, n = 4. *P < 0.05 vs. control. Cmpd C, compound C; IBMX, 3-isobutyl-1-methylxanthine.

Combined effects of submaximal cAMP and AMPK activation. We examined whether increasing cAMP along with activation of AMPK might additively suppress ROS production in high glucose. A 16-h incubation with 0.4 μmol/l forskolin provided a submaximal increase in cellular cAMP content to ~20% of the increase in cAMP observed with 2.0 μmol/l forskolin (Fig. 6). By itself, this small increase in cAMP did not significantly reduce ROS production in high glucose. However, treatment for 16 h with forskolin in doses of 0.4 or 2.0 μmol/l added to cells also treated with AICAR (1 mmol/l) resulted in an additive suppression of the generation of H_2O_2 in high glucose by 17 and 49%, respectively (both $P < 0.05$; data not shown).

Effects of fAd on oxidized LDL-induced ROS production in BAECs. Treatment of BAECs with oxidized LDL (2 μg/ml) increased ROS production by 91% (Fig. 7), similar to the effect of high glucose in BAECs (Fig. 1*B*) and consistent with our previous findings (16). Treatment of BAECs with fAd (3 μg/ml) completely suppressed the increased ROS generation by oxidized LDL in a manner at least partially dependent on PKA because it was suppressed 66% by the PKA-inhibitor Rp-cAMPS (Rp-adenosine 3′,5′-cyclic monophosphorothioate). Further evidence of a potential role for cAMP signaling in the effect of fAd on ROS generation in BAECs was also suggested by treatment with forskolin, which reduced the oxidized LDL-induced increase in ROS generation by 76% (Fig. 7).

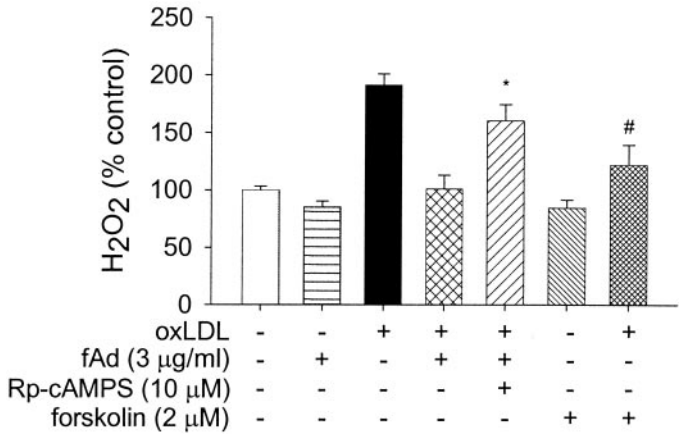

FIG. 7. Effect of fAd and other agents on oxidized LDL-induced ROS production in BAECs. Quiescent BAECs were treated for 15 min with the PKA inhibitor Rp-cAMPS (10 μmol/l) or forskolin (2 μmol/l) or for 30 min with fAd as indicated before adding oxidized LDL (2 μg/ml). The luminol assay for H_2O_2 was performed 16 h later. The data are expressed as the percent of basal H_2O_2 production (control) and represent the means ± SE, $n = 3$. *$P < 0.05$ vs. oxidized LDL and fAd-treated sample; #$P < 0.05$ vs. oxidized LDL-treated sample.

DISCUSSION

In the current study, we found that both the bacterially expressed gAd as well as the fAd from a eukaryotic expression system significantly reduced the generation of H_2O_2 in vascular endothelial cells exposed to high glucose. Interestingly, adiponectin suppression of excess ROS production in high glucose was found to be mediated by a cAMP/PKA-dependent pathway because gAd increased cellular cAMP content, inhibition of PKA blocked the effect of both gAd and fAd to suppress ROS generation, and the suppression of glucose-induced ROS was also mimicked by increasing cellular cAMP. A previous report, examining the suppression of TNF-α–induced inhibitor of κBα phosphorylation and nuclear factor-κB activation by adiponectin in aortic endothelial cells, also showed that the effect of gAd was accompanied by cAMP accumulation and was blocked by inhibitors of adenylate cyclase or PKA (34). Together, these findings help establish the cAMP/PKA pathway as a major signaling system mediating the effects of adiponectin in endothelial cells. Recent studies in other experimental systems have also highlighted the potential involvement of cAMP/PKA signaling in the regulation of ROS production in vascular cell types. Similar to our results presented here for adiponectin, a vasodilator peptide secreted from vascular endothelial cells known as adrenomedullin has also been shown to suppress angiotensin II–induced ROS generation in smooth muscle cells via a cAMP/PKA-dependent mechanism (39). Thus, cAMP signaling might represent a pathway for integrating the regulation of ROS generation from various upstream mediators.

Our current study also calls into question the primacy of AMPK in the endothelial signaling effects of adiponectin, and it suggests that AMPK may work in concert with the cAMP pathway. AMPK is a highly conserved heterotrimeric signaling kinase responsive to hypoxia, exercise, and cellular stress that has been strongly implicated in a variety of cellular responses (40,41). Adiponectin metabolic signaling in liver, skeletal muscle, and adipose cells is mediated by activation of AMPK (17–19). Some actions of adiponectin in vascular endothelial cells, e.g., increased production of NO via increased phosphorylation of endothelial NO synthase at Ser^{1179}, appear to be mediated by adiponectin activation of AMPK (15). Under high-glucose conditions, the tendency toward apoptosis in endothelial cells and a diminished ability of insulin to activate Akt was prevented by activation of AMPK with AICAR (42,43), suggesting that AMPK may also play a key role in protecting endothelial cells from some of the adverse effects of hyperglycemia. Very recently, Kukidome et al. (33) provided evidence that AMPK activation reduces hyperglycemia-induced ROS of mitochondrial origin in HUVECs. Our results have expanded on these findings by showing that activation of AMPK with AICAR partially diminishes glucose-induced ROS production and that the effect of submaximal elevation of cellular cAMP content is further enhanced by activation of AMPK. Because we found that pharmacological inhibition of AMPK did not block the effect of gAd to suppress glucose-induced ROS production, this action of gAd appears to be largely independent of AMPK activity.

Interestingly, precedent does exist to potentially link AMPK signaling and PKA activation. The AMPK kinase LKB1 contains a consensus PKA phosphorylation site, and it is phosphorylated by PKA (44). Glucagon signaling in liver was recently shown to involve the activation of a PKA/LKB/AMPK pathway upstream of mTOR (mammalian target of rapamycin) (45). In the murine preadipocyte 3T3-L1 cell line, cAMP was also shown to activate AMPK, which was required for maximal activation of lipolysis (46). In human granulocytes, cAMP-elevating agents inhibited ROS production by a PKA-dependent mechanism (47). We found in HUVECs that AMPK inhibition did not abrogate the reduction of high-glucose–induced ROS production by gAd, suggesting that the involvement of the PKA pathway in this process was not dependent on AMPK. Furthermore, increasing cAMP levels or blocking PKA activity in HUVECs did not affect the ability of gAd to activate AMPK (K.M., B.J.G., unpublished observations). The cross-talk between cAMP/PKA and AMPK signaling may also be dependent on the cell type under study. Additional work should be done to explore potential interactions between these two important pathways, both of which have now been demonstrated to be involved in adiponectin vascular signaling.

Another provocative aspect of adiponectin's effects in the vasculature is the contrasts that have been observed with its complementary adipokine leptin (48,49). Although both of these ligands activate the AMPK pathway, adiponectin has salutary effects on vascular function, whereas leptin elicits a pathogenic response inducing vascular permeability and can cause nonphysiological vascular remodeling (50–52). However, contrary to the effects we have demonstrated for adiponectin to suppress ROS production, at least some of the detrimental effects of leptin in the endothelium may be mediated by a leptin-induced increase in ROS production (53). Enhanced cellular fatty acid oxidation by leptin has also been linked to PKA signaling in early studies (54), before the identification of a major role for activation of AMPK in leptin metabolic signaling (55). Further work will help sort out the differences in signaling between adiponectin and leptin and how they may interact by an integration of parallel signaling pathways and signal cross-talk (49).

In our previous work, showing that recombinant gAd blocked superoxide generation elicited by oxidized LDL in aortic endothelial cells (16), we also reported that oxidized LDL–induced superoxide generation was completely

blocked by NADPH oxidase inhibition. In contrast, in the current study we found that treatment of HUVECs with the NADPH oxidase inhibitors only partially inhibited the increased H_2O_2 production under high-glucose conditions. These data suggest that cellular NADPH oxidases may contribute at least in part to the increased generation of ROS resulting from incubation in high glucose and also that the mechanism of glucose-induced ROS differs from that of oxidized LDL–induced ROS. The etiology of superoxide generation resulting from high-glucose incubation is complex and may be derived from several cellular sources, including NADPH-dependent oxidases and mitochondrial oxidative phosphorylation (22,56). Very recent and novel studies have also mechanistically linked cellular NADPH oxidase activity with the release of ROS from mitochondrial sources (57).

In summary, determining the mechanism of regulation of endothelial ROS generation by adiponectin may provide insight into its salutary role in the pathogenesis of the vascular complications of diabetes. Studies to date have suggested that endothelial superoxide generation is a complex process involving both mitochondrial sources and cellular NADPH oxidase activity. The vascular actions of adiponectin have also been shown to involve multiple signaling cascades (2). The demonstration in the current work that adiponectin reverses hyperglycemia-associated endothelial ROS generation via a cAMP/PKA-linked pathway provides a new direction to evaluate this important area for the prevention or treatment of the vascular complications of type 1 diabetes.

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