Adiponectin attenuates angiotensin II-induced oxidative stress in renal tubular cells through AMPK and cAMP-Epac signal transduction pathways

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Obesity is an important risk factor for the progression of chronic kidney disease (CKD) independent of blood pressure and diabetes mellitus, although the mechanisms responsible for this relationship have not been fully elucidated (3, 12, 39). Circulating levels of a cytokine secreted by adipose tissue, adiponectin, are reversely related to obesity (1, 9, 26, 40), and recent studies have shown that adiponectin attenuates cardiovascular injury (5, 31, 36). Adiponectin may play a role in maintaining normal podocyte structure (28, 29, 45), but the effect of adiponectin on the kidney is not well understood. We hypothesized that adiponectin would also play a protective role in the kidney and regulate kidney cell response to injury.

Activation of the renin-angiotensin system (RAS) is a key contributor in the progression of CKD (16, 27, 33, 41, 42), and blockade of the RAS limits kidney injury (16, 33, 42, 44). One important mechanism linking the main effectors of RAS, angiotensin II (ANG II), to kidney injury is the activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and increased production of reactive oxygen species (ROS; Refs. 6, 14, 15, 21, 35). Interestingly, adiponectin has been shown to reduce high glucose-induced oxidative stress by suppressing the activity of NADPH oxidase in endothelial cells and this effect is through the activation of AMP-activated protein kinase (AMPK) and cAMP pathways (11, 19, 22, 29, 34). To determine if adiponectin plays a protective role in renal tubular epithelial cells, we first studied adiponectin receptor expression in our primary human tubular epithelial cells and the signal transduction pathways downstream of adiponectin receptors including AMPK and cAMP pathways. We then sought to determine if adiponectin would prevent ANG II-induced activation of NADPH oxidase in kidney tubular cells and to elucidate the signal transduction pathways responsible for any effect.

MATERIALS AND METHODS

Cells. Human renal proximal tubule epithelial cells (Clonetics Cells; Lonza) were cultured in DMEM/F-12 Ham’s medium (Sigma) with 20% fetal bovine serum, epidermal growth factor (10 ng/ml), transferrin (5 μg/ml), insulin (5 ng/ml), hydrocortisone (0.05 μM), penicillin (50 U/ml), and streptomycin (50 μg/ml). Cells were maintained at 37°C, with 5% CO2-95% air. All studies were done with cells between passages 4–7 as previously described (24, 25).

Real-time RT PCR. After reaching full confluence, tubular cells were treated with or without ANG II (0.1 μM) for 18 h. Cells were collected and total RNA was isolated with RNeasy Mini Kit (Qiagen) following the protocol provided by the manufacturer. cDNA was obtained by reverse transcription with QuantiTech Reverse Transcription Kit (Qiagen) and then used for real-time PCR. Real-time PCR was performed with the TaqMan system (Applied Biosystems) according to the manufacturer’s guideline.

Western blot. Antibodies used were as follows: anti-adiponectin receptor 1 (adipoR1; ab70362) and 2 (adipoR2; ab53399) were from Abcam; anti-Na+-K+-ATPase antibody was from Santa Cruz (sc-85628); antibody against phospho-AMPKα (Thr172; no. 2535) was from Cell Signaling; antibody against phospho-actyl-CoA carboxylase (ph-ACC; 07–303) was from Millipore; antibody against β-actin (A5441) was from Sigma; anti-fibronectin (EPS, sc-8422) antibody was from Santa Cruz; and antibody against α-tubulin (302 011) was from DynaTec Systems. For membrane protein isolation, tubular cells were incubated in 60 mm dishes to full confluence and harvested. Cells were lysed with lysis buffer A (1 mM NaHCO3, 5 mM MgCl2, 50 mM TrisHCl, 10 mM EGTA, 2 mM EDTA, 0.86 mM PMSF, 25 μg/ml leupeptin, and 10 mM benzamidine) on ice for 40 min and then centrifuged at 100,000 g at 4°C for 1 h. The supernatant was discarded. Pellets were incubated with lysis buffer B (lysis buffer A supplemented with 1% Triton X-100) on ice for 1 h and then centrifuged at 100,000 g at 4°C for 1 h. The supernatant was kept for Western blot.
Lucigenin enhanced chemiluminescence assay. The final concentrations and the manufacturers of the reagents used were as follows: 100 μM or 1 mM NADPH (Calbiochem); 5 μM lucigenin (Sigma-Aldrich); 0.1 μM human ANG II (Sigma-Aldrich); 50 or 100 ng/ml recombinant full-length human adiponectin (BioVision); 10 μM di-phenyleneiodonium (Sigma-Aldrich); 100 μM apocynin (Calbiochem); 10 μg/ml anti-adipoR1 antibody (ab70362; Abcam); 10 μg/ml anti-adipoR2 antibody (ab77612; Abcam); 10 μg/ml goat IgG (sc-3697; Santa Cruz); 1 mM 5-aminooimidazole-4-carboxamide-1-β-d-ribofuranoside (AICAR; Toronto Research Chemicals); 1 μM compound C (Sigma-Aldrich); 20 μM 4-chlorophenylthio (pCPT)-cAMP (Sigma-Aldrich); 25 μM dibutyryl (db)-cAMP (Sigma-Aldrich); 0.1 mM SQ22536 (Sigma-Aldrich); 10 μM myristoylated PKI (14–22) amide (mPKI, Calbiochem); and 50 μM 8-(4-chlorophenylthio)-2′-O-methyl (8-CPT-2-O-Me)-cAMP (Calbiochem). Renal tubular cells were grown to confluence and then incubated with the appropriate agents as indicated for 18 h before activity assay. Cells were then washed twice with ice-cold PBS, collected, and centrifuged at 18,000 g at 4°C for 1 min. Supernatant was discarded, and 200 μl of PBS were added to each tube. After 15 s of sonication on ice, samples were centrifuged at 18,000 g at 4°C for 20 min. One-hundred microliters supernatant from each sample were mixed with NADPH and lucigenin from which the light emission was measured by FB12 Luminometer (Bethyl Detection Systems). Protein concentration was determined by Bradford assay (Bio-Rad) and used for normalization.

Dihydroethidium stain. Renal tubular cells were seeded on glass cover slides and cultured to 90% confluence. Cells were then treated for 18 h with ANG II (0.1 μM) and/or adiponectin (100 ng/ml). After being washed with PBS, cells were incubated with dihydroethidium (DHE; 2 μM; Invitrogen) at 37°C for 1 h in dark. At the end of incubation, cells were washed twice with PBS then with distilled water. The cover slides were mounted onto glass slides using Dako Faramount Aqueous Mounting Medium (S3025; Dako). Fluorescent images were captured with Zeiss confocal laser-scanning microscope (LSM510; Carl Zeiss). At least six fields (magnification: ×400) were selected for each experimental group, and the fluorescence intensity for each fields was scored semiquantitatively on the scale of 0 to 4 (0 being dark and 4 being the strongest fluorescence) by a trained nephrologist blinded to the experimental conditions.

Cellular PKA activity assay. Cellular PKA activity was measure with PKA kinase activity kit (Enzo Life Science) following the manufacturer’s guide. Whole cell lysate was used and prepared following the manufacturer’s protocol. Briefly, renal tubular cells cultured to 90% confluence in 60-mm dishes were treated with adiponectin (100 ng/ml for 10 min, 2 h, or 16 h) and pCPT-cAMP (20 μM, 10 min). After being washed with ice-cold PBS once, cells were lysed with lysis buffer on ice for 10 min (20 mM MOPS, 50 mM β-glycerophosphate, 50 mM sodium fluoride, 1 mM sodium vanadate, 5 mM EGTA, 2 mM EDTA, 1% Nonident P-40, 1 mM dithiothreitol, 1 mM benzamidine, 1 mM PMSF, and 10 μg/ml leupeptin and apropin). Afterwards, cells were collected and centrifuged at 15,000 g at 4°C for 15 min. Light absorbance at 450 nm was measured with the Thermomax microplate reader (Molecular Devices.). Protein concentrations were determined by Bradford assay (Bio-Rad) and used for normalization.

NF-κB activity assay. The pNF-κB-Luc plasmid was purchased from Stratagene, and the pRL-TK reporter vector was from Promega. Renal proximal tubule epithelial cells were subcultured in six-well plates for 24 h and then transfected with 0.4 μg pNF-κB-Luc plasmid/well and 3.9 ng/well pRL-TK vector/well using Effectene Transfection Reagent (Qiagen). Twenty-four hours after transfection, cells were treated with ANG II (0.1 μM) and/or adiponectin (100 ng/ml) for 18 h and measured for luciferase activity with Dual-Luciferase Reporter Assay System (Promega). Light emission was read by FB12 Luminometer (Bethyl detection systems). Total protein was determined by Bradford assay (Bio-Rad) and used for normalization.

Statistical analysis. All results were presented as means ± SE. Statistical analyses were performed using GraphPad Prism4 (GraphPad Software). One-way ANOVA with Bonferroni posttest was performed for multigroup comparison and unpaired t-test for comparison between two groups. Statistical significance was defined at P < 0.05.

RESULTS

Adiponectin receptors are expressed in human renal tubular cells. To investigate the effect of adiponectin on tubular cells, we first examined adiponectin receptor expression. Real-time RT PCR results indicated that both the adipoR1 and adipoR2 were transcribed in our human renal tubular cells and stimulation by ANG II for 18 h did not significantly alter the mRNA levels of the receptors. Western blot analysis also confirmed protein expression in the cell membrane fraction (Fig. 1).

Adiponectin inhibits ANG II-induced superoxide generation in renal tubular cells. Cellular level of superoxide was first determined by measuring NADPH oxidase activity using the lucigenin-enhanced chemiluminescence assay. As expected, ANG II increased the NADPH oxidase activity compared with control condition (Fig. 2). Adiponectin attenuated the effect of ANG II on NADPH oxidase at a dose of 50 ng/ml and reduced it further at 100 ng/ml (Fig. 2A). Treatment by adiponectin alone did not affect the NADPH oxidase activity in renal tubular cells. The sensitivity of the assay for NADPH oxidase activity was not affected by the concentration of NADPH (Fig. 2B). The specificity of the assay was confirmed with the NADPH oxidase inhibitors diphenyleneiodonium and apocynin (Fig. 2, C and D). The inhibition by adiponectin on ANG II-induced oxidative stress was also examined by DHE fluorescent stain (Fig. 3). In agreement with the result from NADPH oxidase activity assay, adiponectin completely normalized the increase in ROS following ANG II treatment.

Inhibition by adiponectin on ANG II-induced NADPH oxidase activation is adipoR1 dependent. To investigate which receptor for adiponectin was responsible for its antioxidative effect in renal tubular cells, blocking antibodies against adipor1 and R2 were used in combination with ANG II and adiponectin. Cotreatment by anti-adipoR1 but not anti-adipoR2 antibody reversed the effect of adiponectin on NADPH oxidase activation following ANG II stimulation (Fig. 4). Treatment with a nonspecific IgG did not alter the baseline NADPH oxidase activity.

Inhibitory effect of adiponectin on ANG II-induced NADPH oxidase activation in renal tubular cells is mediated by AMPK signaling pathway. Adiponectin attenuates oxidative stress in podocytes and endothelial cells through the AMPK signal transduction pathway (11, 29). To test whether AMPK also mediated the effect of adiponectin on ANG II-induced NADPH oxidase, we first treated the renal tubular cells with the AMPK agonist AICAR (11, 29). Treatment with AICAR alone had no effect on basal NADPH oxidase activity, but AICAR prevented ANG II-induced NADPH oxidase activation (Fig. 5A). We then studied the effect of an AMPK antagonist, compound C. The antagonist completely blocked the inhibitory effect of adiponectin on ANG II-induced NADPH oxidase activation (Fig. 5B). In addition, adiponectin increased phosphorylation of AMPK at Thr-172 as well as phosphorylation of the AMPK target protein ACC at 1 h (Fig. 5C).

cAMP/Epac pathway is also involved in the inhibition of adiponectin on ANG II-induced NADPH oxidase activation. The cAMP pathway was also reported to mediate the antioxidative effects of adiponectin in endothelial cells (11, 22). We
therefore investigated if the cAMP pathway played a role in reducing the ANG II-caused increase in ROS production in renal tubular cells. Treatment with either of the stable cAMP analogs pCPT-cAMP or db-cAMP, recapitulated the effect of adiponectin on ANG II-induced NADPH oxidase (Fig. 6A).

We then studied the effect of an adenylate cyclase inhibitor, SQ22536. This compound blocked the inhibitory effect of adiponectin on ANG II-induced NADPH oxidase activation (Fig. 6B). Increased cellular cAMP levels can signal through PKA and through AMP-activated guanine nucleotide exchange factors (cAMP-GEFs, also known as Epacs; Refs. 13, 18). Using a nonradioactive assay, we measured the level of PKA activity after 10 min, 2 h, or 16 h of adiponectin treatment. Neither short nor long time stimulation increased PKA activity in the renal tubular cells (Fig. 7A). We further tested for a role for PKA by treating the cells with a specific cell-permeable PKA inhibitor, mPKI. mPKI did not inhibit the effect of adiponectin on ANG II-induced NADPH oxidase activation (Fig. 7B). In contrast, the specific Epac activator 8-CPT-2-O-Me-cAMP reduced the activity of NADPH oxidase following ANG II treatment (Fig. 8A). Cross talk between cAMP and AMPK signaling pathways has been demonstrated in hepatocytes (4). We therefore studied the effect of the AMPK inhibitor, compound C, on the inhibition of ANG II-induced NADPH oxidase by the Epac activator 8-CPT-2-O-Me-cAMP (Fig. 8B). Activation of Epac did not inhibit ANG II-induced NADPH oxidase activation in the presence of compound C.

Adiponectin attenuated the inflammatory and fibrotic response induced by ANG II in renal proximal tubular cells. To extend the protective effect of adiponectin in kidney cells beyond ROS production, we studied ANG II-induced activation of NF-κB, a major mediator of the cellular inflammatory response, and the production of fibronectin, an extracellular matrix protein that accumulates in kidney injury. We transfected cells with an NF-κB-reporter construct, and NF-κB activation was determined by measuring luciferase activity. As expected, ANG II caused a robust increase in NF-κB activity in renal tubular cells transfected with the NF-κB reporter construct. This effect was attenuated by adiponectin (Fig. 9). The expression of fibronectin was measured by Western blot analysis of protein lysates from our renal tubular cells. Stimulation with ANG II was associated with increased fibronectin protein generation, and this effect was also inhibited by adiponectin (Fig. 10).

DISCUSSION

The mechanism(s) responsible for the relationship between obesity and progression of CKD have not been fully elucidated, although recent evidence suggests that adipose tissue-derived hormones and cytokines may play a role (3, 12, 39). Circulating levels of leptin rise with obesity and may play a direct role in kidney injury (7, 42). In contrast, circulating levels of adiponectin are negatively correlated with body mass index (1, 9, 26, 40). Although adiponectin impacts on cardiovascular injury, it is unclear if the kidney is also a target organ for adiponectin. In the current study, we focused on the interaction between adiponectin and ANG II, a well-known contributing factor to CKD progression, and specifically on the effect of adiponectin on ANG II-induced production of ROS.

There are two receptors for adiponectin, adiponectin R1 and adiponectin R2 (2, 8, 10, 30), and our first major observation was that both adiponectin receptor subtypes were expressed in our primary human kidney tubular cells. They colocalized with Na⁺/K⁺-ATPase, indicating that they were present on the cell membrane. Adiponectin R1 and adiponectin R2 are seven-transmembrane...
spanning proteins distinctive from G protein-coupled receptors in that the membrane orientation of the NH₂- and COOH terminals is reversed (2, 8, 30). AdipoR1 is widely expressed in human tissues, most abundantly in the liver tissue and skeletal muscle (10). AdipoR2 expression is usually considered to be more restricted to liver and muscle (10). The ligand-binding sites are slightly different for adipoR1 and R2: adipoR1 has higher affinity for globular adiponectin, while adipoR2 has intermediate affinity for both globular and full-length adiponectin (2). In vitro studies have also shown that the receptor subtypes are expressed in podocytes (28). The regulation of expression of adipoR1 and adipoR2 has not been fully characterized (10), and therefore, we studied the effect of ANG II on expression: there was no effect of ANG II on mRNA expression of either receptor in our short-term studies.

We next chose to look at interactions between adiponectin and ANG II because activation of the RAS and increased generation of ANG II contribute to the development and progression of kidney disease (16, 27, 33, 41, 43, 44), and we focused on ANG II-induced activation of NADPH oxidase because oxidative stress links the bioactivity of ANG II to tissue fibrosis and inflammation (6, 14, 15, 21, 35). Our second major observation in this study was that adiponectin inhibited ANG II-induced NADPH oxidase activation and oxidative stress in renal tubular cells in a dose-dependent manner. Adiponectin has been shown to suppress ROS generation and reduce high-glucose-induced oxidative stress in endothelial cells and glomerular podocytes (11, 19, 22, 28, 34), although an effect on ANG II-induced oxidative stress in kidney tubular cells has not been reported previously. Additionally, adiponectin inhibited ANG II-induced activation of NF-κB, an important regulator of cellular inflammation and inhibited ANG II-induced increase in fibronectin expression. These findings suggest that adiponectin may play a protective role in the kidney by modifying oxidative stress and account at least in part for the relationship between obesity and CKD.

The signal transduction pathways linking adiponectin to inhibition of NADPH oxidase may be stimulus and cell specific, at least to the extent that they have been studied (11, 22, 28). With blocking antibodies, we found differential roles for the adiponectin receptors on the antioxidative effect against ANG II in renal tubular cells. Our third major observation was that adipoR1 mediated most if not all of the inhibition on NADPH oxidase activation by adiponectin in renal tubular cells. Binding of adiponectin to its cognate receptors has been

![Fig. 2. Adiponectin inhibited ANG II-induced NADPH oxidase activity in a dose-dependent manner in renal tubular cells. A: after treatment with ANG II (0.1 μM) and/or adiponectin (50 ng/ml or 100 ng/ml) for 18 h, NADPH oxidase activity was measured by lucigenin enhanced chemiluminescence assay. B: after being incubated with ANG II (0.1 μM) for 18 h, renal tubular cells were collected and sonicated. Samples were then mixed with low (100 μM) or high (1 mM)-dose NADPH, and NADPH oxidase activity was measured with a lucigenin chemiluminescence assay. C: increase in NADPH oxidase activity caused by ANG II was attenuated by diphenyleneiodonium (DPI; 10 μM). D: increase in NADPH oxidase activity caused by ANG II was attenuated by apocynin (100 μM). Cells with no treatment were used as the control condition. Results are presented as means ± SE, and at least 3 separate experiments were done in each group. *P < 0.05 vs. control; #P < 0.05 vs. ANG II treated.]
shown to modulate several signal transduction pathways, including AMPK, cAMP, peroxisome proliferator-activated receptor-α, and p38 MAPK (2, 30). We utilized full-length adiponectin and focused on AMPK and cAMP because each of these pathways has been related to the effect of adiponectin on oxidative stress. For example, Sharma (28) demonstrated that inhibition on high-glucose-induced NADPH oxidase activation by adiponectin in podocytes was dependent on AMPK activation. In contrast high-glucose-induced NADPH oxidase activation was cAMP-dependent in endothelial cells (22). Interestingly, Kim et al. (11) showed that adiponectin suppressed ROS generation after palmitate stimulation and that the inhibition was dependent on both cAMP and AMPK signaling. Accordingly, we then sought to define the signal transduction pathways linking adiponectin/adipoR1 to inhibition of ANG II-induced NADPH oxidase activation.

Our next major observation was that the inhibitory effect of adiponectin was dependent on both AMPK and cAMP signal transduction pathways in renal tubular cells. To establish this we looked at the effect of both stimulation and blockade of AMPK and cAMP signaling pathways with pharmacological activators and inhibitors. We used AICAR, an AMPK agonist, and compound C, an AMPK antagonist, to test the AMPK pathway, and we used two stable cAMP analogs, pCPT-cAMP and db-cAMP, and the adenylate cyclase inhibitor SQ22536 to test the cAMP pathway. The activation of each pathway in the renal tubular cells was sufficient to inhibit ANG II-induced NADPH oxidase activation, as was inhibition of each pathway sufficient to block the inhibitory effect of adiponectin on ANG II-induced NADPH oxidase activation. Adiponectin treatment also led to the phosphorylation of AMPK at Thr-172 and an increase in its activity, which was assessed by measuring the phosphorylation of a recognized substrate, ACC, an effect that was abrogated by cotreatment with the AMPK inhibitor compound C.

Interestingly, treatment with adiponectin failed to activate PKA, the classic downstream effector of cAMP in our cells. In accord with this finding, the PKA-specific inhibitor mPKI showed no influence on the effect of adiponectin on ANG II-induced NADPH oxidase activation in renal tubular cells. Taken together these findings suggest that the cAMP/PKA is not linked to the inhibitory effect of adiponectin on ANG II-induced NADPH oxidase activation. This prompted us to examine the effect of another molecule downstream of cAMP,
Epac. Epacs, or exchange proteins directly activated by cAMP, are enriched in renal proximal tubular cells (17) and have been shown to protect proximal tubular cells from cisplatin-induced apoptosis (23) and the kidney from ischemia-reperfusion injury (32). The specific Epac activator 8-CPT-2-O-Me-cAMP inhibited the increase in NADPH oxidase activity after ANG II stimulation, suggesting that Epac is an important molecule for transducing the antioxidative signal from adiponectin and cAMP in renal tubular cells.

The involvement of both the AMPK and the cAMP-Epac pathways in the inhibitory effect of adiponectin on ANG II-induced NADPH oxidase activation suggests that there may be cross talk between the two signal transduction pathways. Previously, Fu et al. (4) found that activation of the cAMP-Epac pathway led to AMPK activation through LKB1 in liver cells. Omar et al. (20) also reported that a specific Epac activator increased AMPK phosphorylation in adipocytes. We therefore studied the ability of the specific Epac activator

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**Fig. 5.** Effect of adiponectin on ANG II-induced NADPH oxidase activation was mediated by AMPK pathway in renal tubular cells. The AMPK agonist 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) reduced the activity of NADPH oxidase after ANG II stimulation similarly as adiponectin (A), while the AMPK antagonist compound C completely blocked the inhibition by adiponectin on ANG II-induced superoxide generation (B). Activation of the AMPK pathway by adiponectin was confirmed by phosphorylation of AMPK at Thr-172 and its target protein ACC (C). ANG II = 0.1 μM; adiponectin = 100 ng/ml; AICAR = 1 mM; compound C = 1 μM. For A and B, cells were incubated with appropriate agents for 18 h. For C, cells were treated as indicated for 1 h, with 30-min preincubation for compound C-treated groups. β-Actin was blotted as a control. Results are presented as means ± SE. For A, n = 3 in each group; *P < 0.05 vs. control; #P < 0.001 vs. ANG II treated. For B, n = 3 in each group; *P < 0.05 vs. control; #P < 0.001 vs. ANG II and adiponectin treated. For C, n = 4 for phospho-AMPK and n = 5 for phosphor-ACC; *P < 0.05 vs. control; !P < 0.05 vs. adiponectin treated.
8-CPT-2-O-Me-cAMP to block ANG II-induced NADPH oxidase activity when AMPK was inhibited by compound C. We found that the inhibitory effect of 8-CPT-2-O-Me-cAMP on ANG II-induced NADPH oxidase activation was completely reversed by compound C. This finding suggests that there is cross talk between cAMP signaling and AMPK signaling in our cells and that Epac plays a key role in this cross talk.

The mechanisms responsible for the inhibition of NADPH oxidase by activation of AMPK and cAMP-Epac have not been fully elucidated, although some studies suggest that inhibition may be mediated by an effect on the expression levels of NADPH oxidase subunits. For example, deletion of the gene for adiponectin is associated with upregulation of expression of the NOX4 in podocytes (28). NOX2 is increased in cardiac tissue in adiponectin knockout mice (34). In addition, Wang et al. (37, 38) discovered that the NADPH oxidase subunits p47phox, p67phox, and NOX2 increased in mice with deletion in the gene for AMPKα2, an effect that may also involve NF-κB. Taken together, these studies suggest that adiponectin negatively regulates NADPH oxidase subunits expression and that this effect may account, at least in part, for the reduction in agonist-induced NADPH oxidase activity by adiponectin.

In summary, our data show that adiponectin receptors are expressed in primary human kidney tubule epithelial cells and that AMPK and cAMP signal transduction pathways are activated by adiponectin in these cells. Adiponectin inhibits ANG II-induced...
NADPH oxidase activation in these cells, an effect that is dependent on adiponectin. Activation of either AMPK or cAMP by adiponectin is sufficient to exert this inhibitory effect. Cross talk between these pathways is mediated in part by Epac. Adiponectin also attenuated the increase in NF-κB activity and fibronectin expression caused by ANG II stimulation. Together, these in vitro findings support the hypothesis that adiponectin may impact progression of CKD by limiting ANG II-induced oxidative stress, inflammation, and fibrosis in the kidney.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

Fig. 8. Epac was responsible for the effect of adiponectin on ANG II-induced NADPH oxidase activation. The specific Epac activator 8-(4-chlorophenylthio)-2'-O-methyl (8-CPT-2-O-Me)-cAMP reduced the activity level of NADPH oxidase following ANG II treatment (A), which was blocked by the AMPK antagonist compound C (B). Renal tubular cells were treated for 18 h before activity measurement. ANG II = 0.1 μM; 8-CPT-2-O-Me-cAMP = 50 μM; compound C = 1 μM (30-min pretreatment). Results are presented as means ± SE; n = 3 for each group. For A, *P < 0.05 vs. control; #P < 0.001 vs. ANG II treated. For B, *P < 0.05 vs. control; #P < 0.001 vs. ANG II and adiponectin treated; !P < 0.05 vs ANG II treated.

Fig. 9. Adiponectin reduced NF-κB activity induced by ANG II. Human renal tubular cells were transfected with pNF-κB-Luc plasmid as described previously. After 18 h of incubation with or without ANG II (0.1 μM) and adiponectin (100 ng/ml), cells were lysed and the lysates were used for luciferase activity assay; n = 3 for all groups. *P < 0.001 vs. transfaction control; #P < 0.001 vs. ANG II-treated group.

Fig. 10. Adiponectin attenuated the increase in fibronectin expression after ANG II stimulation in renal tubular cells. Renal proximal tubule cells were incubated with ANG II (0.1 μM) and/or adiponectin (100 ng/ml) for 18 h before harvested for fibronectin immunostaining. α-Tubulin was used as loading control. Results are shown as means ± SE, and n = 4 for each group. *P < 0.05 vs. control; #P < 0.05 vs. ANG II and adiponectin treated.

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