Effect of simvastatin on high glucose- and angiotensin II-induced activation of the JAK/STAT pathway in mesangial cells

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Banes-Berceli, Amy K., Sean Shaw, Guochuan Ma, Michael Brands, Douglas C. Eaton, David M. Stern, David Fulton, R. William Caldwell, and Mario B. Marrero. Effect of simvastatin on high glucose- and angiotensin II-induced activation of the JAK/STAT pathway in mesangial cells. Am J Physiol Renal Physiol 291: F116–F121, 2006. First published January 31, 2006; doi:10.1152/ajprenal.00502.2005.—In the current study, we investigated the effect of simvastatin on the ability of high glucose (HG) and ANG II to activate the JAK2-STAT signaling cascade and induce glomerular mesangial cell (GMC) growth. We found that pretreatment with simvastatin significantly inhibited HG- and ANG II-induced collagen IV production, JAK2 activation, and phosphorylation of STAT1 and STAT3 in GMC. We also found that the activation of JAK2 by HG and ANG II was dependent on the Rho family of GTPases. Consistent with these in vitro results, both albumin protein excretion and phosphorylation of JAK2, STAT1, and STAT3 were attenuated in renal glomeruli by administration of simvastatin in a streptozotocin-induced rat model of HG diabetes. This study demonstrates that simvastatin blocks ANG II-induced activation of the JAK/STAT pathway in the diabetic environment, in vitro and in vivo, and, thereby, provides new insights into the molecular mechanisms underlying early diabetic nephropathy.

diabetes; Rho GTPase; nephropathy

THE 3-HYDROXY-3-METHYLGLUTARYL-COENZYME A (HMG-CoA) REDUCTASE INHIBITORS, OR STATINS, ARE POTENT INHIBITORS OF CHOLESTEROL SYNTHESIS THAT ARE EXTENSIVELY USED IN THE TREATMENT OF HYPERCHOLESTEROLEMIA (11, 12). SEVERAL STUDIES HAVE DEMONSTRATED THAT STATINS REDUCE CARDIOVASCULAR MORBIDITY AND MORTALITY (9, 13). FURTHERMORE, STATINS HAVE ALSO BEEN POSTULATED TO CONFER RENOPROTECTION IN A VARIETY OF GLomerular diseases INCLUDING DIABETIC NEPHROPATHY (6). IT IS USUALLY ASSUMED THAT THE BENEFICIAL EFFECTS OF STATINS RESULT FROM THE COMPETITIVE INHIBITION OF CHOLESTEROL BIOSYNTHESIS. HOWEVER, STUDIES HAVE DEMONSTRATED THAT STATINS MAY ALSO EXERT ADDITIONAL BENEFICIAL EFFECTS BEYOND THEIR CHOLESTEROL-LOWERING PROPERTIES BY PREVENTING THE SYNTHESIS OF ISOPRENOID INTERMEDIATES, SUCH AS FARNESYL PYROPHOSPHATE (FPP) AND GERANYLGERANYL PYROPHOSPHATE (GGPP), WHICH FORM ADDUCTS WITH A RANGE OF INTRACELLULAR SIGNALING MOLECULES SUCH AS RHO, RAC, AND RAS (5, 6). RHO, RAC, AND RAS ARE MONOMIC G PROTEINS WITH MOLECULAR MASSES OVER THE RANGE 20–30 KDA, WHICH FUNCTION AS MOLECULAR SWITCHES TO CONTROL MANY EUKARYOTIC CELL FUNCTIONS LIKE GROWTH AND PROLIFERATION OF RENAL GLOMERULAR MESANGLIAL CELLS (GMC) (5, 6). SEVERAL STUDIES HAVE PROVIDED EVIDENCE OF CROSSTALK BETWEEN G PROTEIN-COUPLED RECEPTORS (GPCR) AND SMALL G PROTEINS, AND IT IS WELL DOCUMENTED THAT GPCR SIGNALING THROUGH HETEROTrIMERIC G PROTEINS CAN LEAd TO THE ACTIVATION OF RAC, RAS, AND RHO GTPASEs (5, 6). THEREFORE, INHIBITION OF Rho, RAS, AND RAC, WHOSE PROPER MEMBRANE LOCALIZATION AND FUNCTION ARE DEPENDENT ON ISOPRENYLYATION, MAY PLAY AN IMPORTANT ROLE IN MEDIATING THE PLEIOTROPIC EFFECTS OF STATINS (5, 6).

A RECENT STUDY DEMONSTRATED THAT STATINS PREVENT ANG II-INDUCED CELLULAR AND ORGAN DAMAGE, SUCH AS THE PROLIFERATION OF AORTIC VASCULAR SMOOTH MUSCLE CELLS (VSMC) AND CARDIAC HYPERTROPHY, BY BLOCKING ACTIVATION OF THE JAK/STAT SIGNALING PATHWAY (7). WE PREVIOUSLY SHOWED THAT HIGH GLUCOSE (HG) AUGMENTS ANG II-INDUCED ACTIVATION OF JAK2 IN GMC AND VSMC (1, 2, 15). IN ADDITION, WE FOUND THAT HG ALTERS THE ACTIVATION OF THE JAK/STAT PATHWAY IN VIVO THROUGH ANG II IN RAB KINDNEY GLOMERULAR BY INDUCING PHOSPHORYLATION OF JAK2 KINASE AND STAT1 AND STAT3 PROTEINS (3). IN STREPTOZOTOCIN (STZ)-INDUCED DIABETIC RATS, ADMINISTRATION OF THE JAK2 INHIBITOR AG490 REDUCED BOTH SYSTOLIC BLOOD PRESSURE AND URINARY PROTEIN EXCRETION (3). TO OUR KNOWLEDGE, THESE ARE THE FIRST OBSERVATIONS IDENTIFYING THE INCREASED ACTIVATION OF JAK2 IN AN IN VIVO MODEL OF GLOMERULAR DYSFUNCTION.

IN THE CURRENT STUDY, OUR GOAL IS TO ELUCIDATE THE MECHANISMS BY WHICH SIMVASTATIN HIBITS THE ACTIVATION OF JAK2 IN RESPONSE TO HG AND ANG II. ONCE ACTIVATED, JAK2 PHOSPHORYLATES AND ACTIVATES THE STAT TRANSCRIPTION FACTORS, WHICH, IN TURN, INDUCE PRODUCTION OF THE EXTRACELLULAR MATRIX (ECM) PROTEIN COLLAGEN IV IN GMC SUCH PROMOTING THE DEVELOPMENT OF DIABETIC NEPHROPATHY (1). WE, THEREFORE, PREDICT THAT STATINS MAY INHIBIT GMC SYNTHESIS OF ECM BY ANTAGONIZING HG- AND ANG II-INDUCED ACTIVATION OF THE JAK/STAT SIGNALING CASCADE. THE CURRENT STUDY TESTS THIS CONCEPT IN BOTH CELL CULTURE AND IN VIVO IN AN STZ-INDUCED RAT MODEL OF TYPE 1 DIABETES.

MATERIALS AND METHODS

Isolation and culture of GMC. GMC were obtained from isolated, collagenase-treated rat glomeruli as previously described (1). In brief, glomeruli were harvested from male 275- to 300-g Sprague-Dawley rats by filtration with ice-cold 0.9% NaCl solution through a 200-, 150-, 120-, and 50-mm nylon mesh. Those retained on the sieve were collected, washed by centrifugation (4°C; 2,000 g), and incubated with 250 U/ml collagenase (type I) for 30 min at 37°C under constant, gentle shaking. GMC were plated on plastic tissue culture flasks in
DMEM (pH 7.4) in normal glucose (NG; 5.5 mM) or HG (25 mM) or NG plus mannitol (19.5 mM). Culture medium was supplemented with 17% (vol/vol) fetal bovine serum, 100 U/ml penicillin, 100 µg/ml streptomycin, 0.5 U/ml insulin, and 10 µl/ml of Fungizone. Cells were incubated at 37°C in humidified 5% CO2-95% air. The culture media was not changed for the first 4 days. Thereafter, the medium was changed every other day until confluence. Subsequently, cells were subcultured at a 1:6 split ratio at 7-day intervals, and the medium was changed at 2-day intervals. GMC, passages 1–6, were grown to 75–85% confluence, washed once with serum-free DMEM, and growth-arrested in serum-free DMEM in NG for 24 h to synchronize the cell growth. After this time period, the media was changed to fresh serum-free media containing NG, HG, or NG plus mannitol in the presence or absence of 5 µM simvastatin for 24 h before ANG II stimulation.

**Activation and preparation of simvastatin.** Simvastatin was prepared by opening the lactone ring and activating it. Briefly, we took 42 mg of the native simvastatin and dissolved it in 1 ml of 95% ethanol. We then added 0.15 ml of 0.1 N NaOH and after heating at 50°C for 2 h, we then took the resulting solution and neutralized it with HCl to a pH of ~7.2. We then brought up the solution to a volume of ~3 ml with distilled water. Following freeze drying, the open ring form of simvastatin was then dissolved in distilled water to a concentration of 100 mM and aliquots were stored frozen (~80°C) until use for both the in vivo or in vitro studies.

**Activation of JAK and STAT proteins.** To identify the phosphorylation state of JAK2 and the STAT proteins, serum-starved GMC grown in either NG (5.5 mM glucose) or HG (25 mM glucose) pretreated with either geranylatedfarnesyltransferase cell-permeable inhibitor (GGTI) GGTI-286 or farnesyltransferase cell-permeable inhibitor (FTI) Manumycin A or simvastatin for 24 h in the presence or absence of GTPγS or FPP. After all the pretreatments indicated, GMC were stimulated with ANG II (100 nM) for the times indicated. At the end of the stimulation period, cells were washed twice with ice-cold PBS-V (PBS with 1 mM/l Na2VO4). Each dish was then treated for 60 min with ice-cold lysis buffer (20 mM/l Tris·HCl, pH 7.4, 2.5 mM/l EDTA, 1% Triton X-100, 10% glycerol, 1% deoxycholate, 0.1% SDS, 10 mM/l Na2PO4, 50 mM/l NaF, 1 mM/l Na3VO4, and 1 mM/l PMSF), and the supernatant fraction was obtained as cell lysate by centrifugation at 58,000 x g for 25 min at 4°C. Protein concentration for each sample was assessed by a modification of the Bradford method (1, 2).

Subsequently, samples were resolved by SDS-PAGE (10%), transferred to nitrocellulose membranes, and blocked by a 60-min incubation at room temperature (22°C) in TTBS (TBS with 0.05% Tween 20, pH 7.4) plus 5% skimmed milk powder. Nitrocellulose membranes were incubated overnight at 4°C with affinity-purified anti-phosphospecific JAK and STAT antibodies (namely JAK2, and STAT1, STAT3, respectively). Subsequently, nitrocellulose membranes were washed twice for 10 min each with TTBS and incubated for varying times with goat anti-rabbit IgG horseradish peroxidase conjugate. After extensive washing, bound antibody was visualized on Kodak Biomax film using a Pierce Supersignal substrate chemiluminescence detection kit. Molecular weight markers were electrohoresed simultaneously with samples.

**Collagen IV ELISA.** Type IV collagen was measured by competitive ELISA (1) using type IV collagen from an Engelbreth-Holm-Swarm (EHS) tumor as the standard, rabbit anti-mouse type IV collagen as the primary antibody (both from Collaborative Research, Medford, MA), and horseradish peroxidase-conjugated goat anti-rabbit IgG (Bio-Rad, Hercules, CA) as the secondary antibody. The assay was performed on media collected at the end of the experimental incubation period.

**STZ-induced diabetes.** All studies were conducted with the approval of the Medical College of Georgia animal care and use committee in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male Sprague-Dawley rats (225–250 g) from Harlan Laboratories (Indianapolis, IN) were rendered diabetic by a single intravenous injection of streptozotocin (STZ; 50 mg/kg) made up in fresh 0.1 M citrate buffer, pH 4.5 (3). Rats were given STZ via the tail vein. Age-matched control rats received buffer only. The diabetic state was confirmed 72 h later by measurement of tail blood glucose (BG) level using the Accu-check glcometer. All rats given STZ had a blood glucose concentration exceeding 15 mM and thus were considered diabetic.

Two groups of STZ-treated rats were studied: a control diabetic group and a group treated with simvastatin (5 mg·kg⁻¹·day⁻¹) via Alza miniosmotic pumps implanted subcutaneously (n = 12 each). All rats in these experiments were treated daily with 2 U of insulin (Humulin, Eli Lilly, Indianapolis, IN) administered subcutaneously (sc) to prevent ketoacidosis. Animals were fed standard Purina rat chow (Ralston Purina, Richmond, IN), had free access to tap water ad libitum, and were kept on a 12:12-h light-dark cycle.

**Isolation of glomeruli.** Glomeruli were harvested as previously described (3). Briefly, 4 wk following STZ or control buffer injection, rats were anesthetized with pentobarbital sodium and kidneys were removed, decapitated, and placed in ice-cold 0.9% NaCl solution.

Kidneys were separated into cortex and medulla, and cortical tissue was minced with a razor blade and pressed against a 0.3-mm stainless steel grid or a 150-µm nylon mesh filter. Large, fibrous tissues were retained on the grid surface, whereas glomeruli and tubular segments passed through. The glomeruli were isolated by filtration through a 70-μm nylon mesh in ice-cold 0.9% NaCl solution. Material retained on the sieve was collected, washed by centrifugation (4°C, 2,000 g), and suspended in 50 mM Tris·HCl (pH 7.4). Tissue was maintained at 4°C during the entire isolation procedure. The purity of the glomerular suspensions was assessed by light microscopy; final preparations were estimated to be at least 95% glomeruli. Glomerular suspensions were homogenized for 1 min in a Polytron (setting 7), centrifuged at 40,000 g for 20 min, and resuspended for 60 min with ice-cold lysis buffer (20 mM Tris·HCl, pH 7.4, 2.5 mM EDTA, 1% Triton X-100, 10% glycerol, 1% deoxycholate, 0.1% SDS, 10 mM Na2PO4, 50 mM NaF, 1 mM Na3VO4, and 1 mM PMSF). The supernatant fraction was obtained by centrifugation at 58,000 g for 25 min at 4°C. Protein concentration for each sample was assessed by modification of the Bradford method (1–3).

**Urine albumin analysis.** Urine albumin concentrations were determined as described previously (3).

**Chemicals.** Molecular weight standards, acrylamide, SDS, N,N'-methylene-bisacrylamide, N,N,N',N'-tetramethylethylenediamine, protein assay reagents and nitrocellulose membranes were purchased from Bio-Rad Laboratories, and collagenase type I was from Worthington Biochemical (Freehold, NJ). Protein A/G-agarose was obtained from Santa Cruz Biotechnology (Santa Cruz, CA), and DMEM, fetal bovine serum, trypsin, and all medium additives were obtained from Mediatech (Herndon, VA). Monoclonal antibodies to phosphoryrosynine (PY20), JAK2, STAT1, and STAT3 were procured from Transduction Laboratories (Lexington, KY). Anti-phosphoryrosynine antibodies for STAT1 and STAT3 were purchased from New England Biolabs (Beverly, MA), and anti-phosphosertine STAT1 and STAT3 antibodies were acquired from Upstate Biotechnology (Lake Placid, NY). Anti-phosphoryrosynine JAK2 antibody was obtained from Biosource International (Camarillo, CA). The Pierce Supersignal substrate chemiluminescence detection kit was obtained from Pierce (Rockford, IL). Goat anti-mouse IgG and anti-rabbit IgG were acquired from Amersham (Princeton, NJ). Simvastatin, Tween 20, ANG II, geranylatedfarnesyltransferase cell-permeable inhibitor GGTI-286 and farnesyltransferase cell-permeable inhibitor (FTI) Manumycin A were all obtained from Calbiochem (La Jolla, CA). All other chemicals was purchased from Sigma (St. Louis, MO).

**Statistics.** Quantitation of band density was performed using NIH Image (Scion USA). Band density is reported in arbitrary densitometry units and analyzed with a two-way ANOVA and a Student-Newman-Kuels post hoc test. In vivo data were analyzed with a

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one-way ANOVA and a Student-Newman-Kuels post hoc test. Data are reported as means ± SE for the number of animals and for the number of samples indicated. Values were considered significant when \( P < 0.05 \).

RESULTS

Effect of simvastatin and isoprenoids on HG- and/or ANG II-induced JAK/STAT signaling pathway activation. Our group recently showed that HG augments the ANG II-induced activation of the JAK/STAT signaling pathway in both GMC and VSMC, which leads to growth and proliferation in both cell types (1, 2, 15, 16). In this study, we focused on JAK2 activation in GMC. As shown in Fig. 1, pretreatment of GMC with simvastatin (5 \( \mu \)M) for 24 h significantly inhibited JAK2 activation induced by HG and ANG II (100 nM) for the times indicated. We also examined whether simvastatin decreased both HG- and ANG II-mediated activation of the STATs. Pretreatment with simvastatin significantly inhibited both HG- and ANG II-mediated tyrosine and serine phosphorylation of STAT1 (Fig. 2) and STAT3 (Fig. 3). Although the serine phosphorylation of STAT1 (Fig. 2) in the presence of ANG II and NG/HG was completely blocked by simvastatin, STAT1 tyrosine phosphorylation was more persistent (Fig. 2). ANG II-induced tyrosine phosphorylation of STAT1 in NG was completely blocked by simvastatin (Fig. 2). However, in HG, ANG II-induced tyrosine phosphorylation of STAT1 was attenuated, but not completely blocked, by simvastatin (Fig. 2). The serine and tyrosine phosphorylation of STAT3 in NG conditions were blocked completely by simvastatin (Fig. 3). The same was true of tyrosine phosphorylation of STAT3 in HG. However, serine phosphorylation of STAT3 in HG in the presence of ANG II was attenuated, but not completely blocked, by simvastatin (Fig. 3). Total protein levels of JAK2, STAT1, and STAT3 were not changed in the presence of these treatments (Figs. 1B, 2B, and 3B).

As previously stated, recent studies also showed that statins exhibit additional beneficial effects beyond their cholesterol-lowering properties by preventing the synthesis of isoprenoid intermediates, such as FPP and GGPP (5, 6). Therefore, to determine the role of these two isoprenoids derived from mevalonate in regulating the inhibitory effect of simvastatin on both the HG- and ANG II-induced activation of JAK2, both HG- and ANG II-stimulated GMC were cotreated with simvastatin (5 \( \mu \)M) in the presence or absence of GGPP (10 \( \mu \)M) or FPP (10 \( \mu \)M). As shown in Fig. 4, cotreatment of cells with GGPP but not with FPP reversed the inhibitory effect of

![Fig. 1. Effect of simvastatin on the high glucose (HG)- and ANG II-induced activation of JAK2 in glomerular mesangial cells (GMC). GMC were incubated in fresh serum-free medium containing either NG, HG, or NG plus mannitol (data not shown) in the presence/absence of simvastatin (5 \( \mu \)M) for 24 h before ANG II stimulation. Immunoblotting on cell extracts was then performed for pJAK2 and total JAK2, as described. Results are representative of 3 experiments. A: normal glucose. B: HG.](http://ajprenal.physiology.org/)

![Fig. 2. Effect of simvastatin on the HG- and ANG II-induced activation of STAT1 in GMC. The same experimental protocol was performed as in Fig. 1, except antibodies to phosphotyrosine (pY) STAT1 and phosphoserine (pS) STAT1 were employed. Results are representative of 3 experiments. A: normal glucose. B: HG.](http://ajprenal.physiology.org/)

![Fig. 3. Effect of simvastatin on the HG- and ANG II-induced activation of STAT3 in GMC. The same experimental protocol was performed as in Fig. 1, except antibodies to phosphotyrosine STAT3 and phosphoserine STAT3 were employed. Results are representative of 3 experiments. A: normal glucose. B: HG.](http://ajprenal.physiology.org/)
Simvastatin and JAK2-STAT Signaling in GMC

A. Control
B. Simvastatin
C. Simvastatin + FPP
D. Simvastatin + GGPP

Fig. 4. Effects of farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) on the simvastatin inhibition on both the HG- and ANG II-induced activation JAK2 in GMC. GMC were incubated in fresh serum-free medium containing either NG, HG, or NG in the presence of simvastatin (5 μM) for 24 h with or without 10 μM FPP and 10 μM GGPP before ANG II stimulation. Immunoblotting on cell extracts was then performed for pJAK2 and total JAK2, as described. Results are representative of 3 experiments.

Fig. 5. Effects of FPP and GGPP inhibitors on the HG- and ANG II-induced activation JAK2 in GMC. GMC were incubated in fresh serum-free medium containing either NG, HG, or NG in the presence of simvastatin (5 μM) for 24 h with or without 10 μM FPP and 10 μM GGPP before ANG II stimulation. Cell extracts were then immunblotted for pJAK2 and total JAK2, as described. Results are representative of 3 experiments.

Fig. 6. Effect of simvastatin on the HG- and ANG II-induced production of collagen IV by GMCs. GMC were preincubated for 24 h in the presence/absence of simvastatin (5 μM) and then exposed to NG or HG medium in the presence/absence of ANG II (100 nM) for an additional 48 h. Collagen IV production was assessed as described in MATERIALS AND METHODS. Results represent the means ± SE of 4 independent cultures and are expressed as nanograms of collagen IV per cell protein content. ANG II induced a significant increase in collagen IV synthesis in both NG (*P < 0.01) and HG (+P < 0.01) conditions compared with control levels. This ANG II-induced production of collagen IV was significantly inhibited by simvastatin in NG (**P < 0.01) and HG (###P < 0.01). HG alone also induced a significant increase (+P < 0.01) in collagen IV synthesis that was significantly inhibited (#P < 0.05) by simvastatin.

Effect of simvastatin on HG- and ANG II-induced production of collagen IV. Previously, we reported that ANG II induced the production of collagen IV in GMC incubated in either NG or HG media. We found that when GMC were incubated in medium alone for 48 h, baseline concentrations of collagen IV were 395 ng/mg cell protein for GMC grown in NG media and 825 ng/mg cell protein for GMC grown in HG media (1). These results indicate that collagen IV was constitutively produced by GMC and that HG induced a significant increase in its synthesis. We also found that incubation of GMC with ANG II for 48 h increased production of collagen IV in a dose-dependent manner, with the peak concentration observed at 100 nM (1). In addition, we have also identified that JAK2 was necessary for both HG- and ANG II-induced collagen IV protein production (1). In this study, we found that preincubating GMC for 24 h with 5 μM simvastatin significantly inhibited both the ANG II- and HG-induced collagen IV protein synthesis in GMC (Fig. 6). Thus signaling events downstream of HMG-CoA reductase are required by ANG II and the HG to induce the synthesis of collagen IV.

Effect of simvastatin on the diabetes-induced activation of JAK and STAT proteins and protein excretion in vivo. We previously documented that both HG and ANG II have the ability to activate both JAK and STAT proteins through the AT1 receptor in GMC both in vitro and in vivo (1, 3). Therefore, we investigated the in vivo effect of simvastatin on the phosphorylation of JAK/STAT in the glomeruli of diabetic rats. Glomeruli were isolated from STZ-treated rats 4 wk after induction of diabetes. For these studies, we examined the tyrosine phosphorylation of JAK2, and both tyrosine and serine phosphorylation of STAT1 and STAT3. In control animals (C; nondiabetic), the phosphorylation of JAK2, STAT1, and STAT3 was minimal (Fig. 7). In contrast, in the diabetic state (S), the phosphorylation of JAK2, STAT1, and STAT3 was significantly elevated (Fig. 7). Treatment of diabetic animals with simvastatin (SS) strongly suppressed the phosphorylation...
of these transcription factors in glomeruli and while the pSTAT1 band was completely inhibited, there was strong attenuation of immunoreactive bands corresponding to pJAK2 and pSTAT3 (Fig. 7). These results indicate that simvastatin has a prominent effect on hyperglycemia-induced activation of JAK2 and the STAT1 and STAT3 transcription factors in vivo. Finally, in the STZ-treated diabetic rat, concurrent treatment with simvastatin significantly reduced albumin protein excretion (Fig. 8). Albumin protein excretion is an early sign of kidney damage associated with diabetes (3). These results, therefore, demonstrate that simvastatin also blocks the ANG II-induced activation of the JAK/STAT pathway and glomerular damage in the diabetic environment in vivo.

**DISCUSSION**

ANG II- and HG-induced GMC dysfunction appears to be directly linked to inflammation and ECM deposition (6, 8). The glomerulus is a key regulator of renal and overall metabolic homeostasis (3). In diabetes, high levels of glucose impact GMC function through many mechanisms. One key mechanism involves the activation of the JAK/STAT pathway (1, 3, 16). For example, we previously reported that exposure of GMC to HG induces the tyrosine phosphorylation of JAK2, which is accompanied by the tyrosine and/or serine phosphorylation of STAT1 and STAT3 (1). The activation of JAK2 was essential for both ANG II and hyperglycemia-induced collagen IV production and GMC growth (1). Furthermore, we also demonstrated that the activation of JAK2 and STAT1 proteins was a requirement for the HG-induced production of TGF-β and fibronectin in GMC (16). We also recently reported that HG stimulated the glomerular phosphorylation of JAK2, STAT1, and STAT3 in vivo and that phosphorylation was reduced in rats treated with the AT1 receptor blocker candesartan and the JAK2-specific inhibitor AG-490 (3). Finally, we also reported that both candesartan and AG-490 inhibited STZ-induced increases in urinary protein excretion (3). These results indicate that the JAK/STAT pathway plays a very important role in the GMC growth and ECM deposition that occurs during diabetic nephropathy. In the current study, we examined the effect of the HMG-CoA reductase inhibitor, simvastatin, on the activation of JAK2, STAT1, and STAT3, in parallel with its effect on collagen production in GMC in vivo and protein excretion in vivo.

Statins, including simvastatin, have been shown to prevent or reverse injury in a variety of cardiovascular and renal diseases (5, 6, 10). In addition to their well-established action in lowering cholesterol levels, statins can also prevent or reverse vascular dysfunction by a variety of mechanisms. For example, statins reduce production of ROS, mediators of inflammation, and enhance physiological nitric oxide (NO) production through increased expression and activity of endothelial nitric oxide synthase (eNOS) (4). More specifically, statins have been shown to improve endothelial function in insulin-sensitive and -resistant diabetes by increasing both eNOS expression and reducing oxidative stress (4, 17). Therefore, it is increasingly clear that the protective effects of statins are not confined to their cholesterol-lowering properties (4, 6).

In this study, we demonstrated that simvastatin suppresses HG/ANG II-induced phosphorylation of JAK2, STAT1, and STAT3 in GMC cell cultures, and in glomeruli from diabetic rats. In addition, we found that simvastatin decreases the production of collagen IV in GMC exposed to HG alone or HG + ANG II. Similarly, simvastatin-treated diabetic rats also displayed a reduction in protein excretion, which is indicative of early kidney damage. Thus activity of HMG-CoA reductase is intimately tied to the activation of JAK/STAT pathways in the diabetic kidney. Furthermore, addition of GGPP reversed the inhibitory effect of simvastatin on JAK2 activation, while incubation with the GGTPase inhibitor mimics the effect of simvastatin suggesting that the simvastatin effect is geranylgeranyl dependent in GMC. In support of our results, two recent studies by Danesh and colleagues (5, 18) explored the modulatory effects of simvastatin and geranylgeranylation on both the HG- and ANG II-induced growth of GMC. They report that cotreatment of cells with simvastatin inhibited both HG- and ANG II-induced growth and proliferation of GMC.
and that the addition of GGPP reversed the inhibitory effect of simvastatin, suggesting that the effect of simvastatin is geranylgeranyl dependent. These observations plus ours in combination with our previous studies demonstrate a direct link between the activation of JAK2/STAT1 and 3, and HG- and ANG II-induced overproduction of ECM in GMC in vitro and in vivo (1, 3, 16). In conclusion, our study provides strong evidence that simvastatin modulates the detrimental effects of HG/ANG II in diabetic kidney glomeruli both in vitro and in vivo by preventing the HG- and ANG II-induced geranylgeranyl-dependent activation of Rac or/and Rho and thereby blocking the activation of JAK2 and both STAT1 and STAT3. Therefore, novel therapies based on the inhibition by statins of the ANG II-induced activation of JAK2 may be a route for future treatment of diabetic nephropathy.

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