Antioxidant Inhibition of Protein Kinase C–Signaled Increases in Transforming Growth Factor-Beta in Mesangial Cells

R.K. Studer, P.A. Craven, and F.R. DeRubertis

Protein kinase C (PKC)-signaled increases in transforming growth factor β (TGFβ) have been implicated in the stimulation of matrix protein synthesis induced by high concentrations of glucose, thromboxane, angiotension II (AII), and other stimuli in cultured glomerular mesangial cells. In the present study, the effects of several antioxidants on mesangial cell responses to high glucose, thromboxane, and AII were examined. α-Tocopherol blocked increases in PKC, TGFβ bioactivity, collagen, and/or fibronectin synthesis induced in mesangial cells by high glucose, the thromboxane analog U46619, and AII. By contrast, α-tocopherol did not alter increases in matrix protein synthesis in mesangial cells in response to exogenous TGFβ, a cytokine that does not activate PKC in mesangial cells and whose actions to stimulate matrix protein synthesis in these cells are not blocked by PKC inhibition or downregulation. Taurine and *N*-acetylcysteine similarly inhibited activation of PKC and increases in TGFβ in response to high glucose, U46619, and AII. α-Tocopherol but not taurine or *N*-acetylcysteine partially blocked increases in PKC activity in mesangial cells in response to the diacylglycerol (DAG) analog, phorbol dibutyrate (PDBu). Thus, α-tocopherol may have direct effects on interaction of the PKC system of mesangial cells with DAG that are not shared by *N*-acetylcysteine or taurine. Increases in TGFβ have been implicated in the pathogenesis of glomerulosclerosis in diabetes and other nephropathies. The capacity of antioxidants to block increases in TGFβ in mesangial cells in response to high glucose, thromboxane, and AII suggests their potential therapeutic utility to attenuate glomerulosclerosis.

EXPANSION OF THE GLOMERULAR mesangium is a characteristic feature of diabetic nephropathy that correlates with the loss of glomerular function[1] and is due, at least in part, to enhanced matrix protein synthesis.[2] In vivo, multiple factors including hyperglycemia per se may interact to mediate this pathological change. Studies of isolated glomeruli in experimental diabetes[3] and in cultured glomerular mesangial cells have implicated the activation of protein kinase C (PKC) by high concentrations of glucose and other PKC agonists as a signal for increased matrix protein synthesis.[4-9] One consequence of PKC activation in mesangial cells is an increase in transforming growth factor beta (TGFβ) bioactivity,[7,10-12] a cytokine that has been linked to the development of glomerulosclerosis in experimental and human models of renal disease, including diabetes.[13-15] In mesangial cells, activation of PKC by high glucose, thromboxane, low-density lipoproteins, or phorbol esters signals increases in TGFβ bioactivity, a step that is in turn critical to the increases in matrix protein synthesis induced by these agents.[7,11,12]

Oxidant mechanisms have been implicated in PKC activation.[16-18] Recent studies have demonstrated that α-tocopherol blocks activation of PKC induced by high glucose in vascular smooth muscle cells (VSMCs), retinal endothelial cells, and isolated glomeruli in vitro.[19-21] Similarly, treatment with α-tocopherol prevents the increases in PKC activity otherwise observed in the glomerulus, retina, and aorta of the streptozotocin-diabetic rat. The relationship between these actions of α-tocopherol and its antioxidant activity remain uncertain.[19] However, PKC-dependent actions of angiotensin II (AII) in myocardial cells are blocked by the antioxidant *N*-acetylcysteine, consistent with involvement of oxidant mechanisms in this signaling pathway.[22] In mesangial cells, α-tocopherol has also been reported to prevent increases in lipid peroxidation, collagen production, and advanced glycosylation products and to reverse the antiproliferative effects of high glucose.[23,24] The current studies were designed to evaluate the effects of α-tocopherol and other antioxidants on the actions of high glucose and thromboxane to activate PKC, increase TGFβ, and stimulate matrix protein synthesis in mesangial cells. Evidence is presented that α-tocopherol and other antioxidants block increases in matrix protein synthesis in mesangial cells induced by high glucose and thromboxane by inhibiting activation of PKC and PKC-signaled increases in TGFβ.

MATERIALS AND METHODS

Mesangial Cell Culture

Mesangial cells were cultured from collagenase-treated glomerular cores of the kidneys of 120- to 150-g female Sprague-Dawley rats as previously reported.[5] They were maintained in RPMI 1640 medium containing 10% fetal calf serum (FCS), 100 U/mL penicillin, 100 μg/mL streptomycin, 5 μg/mL insulin, 5 μg/mL transferrin, and 5 ng/mL sodium selenite at 37°C in 5% CO_2 and 95% air unless otherwise indicated. Viability as assessed by trypan blue exclusion was greater than 95% under all conditions of incubation used. Where indicated in the text, mesangial cells were cultured in media containing 5 or 30 mmol/L glucose for the times indicated prior to study. Media were changed to fresh media of the same composition every 2 to 3 days, and cells were passaged once per week or when the cultures were 80% confluent. Mesangial cells were grown to 80% confluence in 12- or 24-well plates and rendered quiescent by incubation with medium of the same composition already described containing 0.5% FCS for the times indicated in the text before addition of test agents.

PKC Activity

PKC activity of mesangial cells was determined in situ as previously described.[5] Thromboxane activation of PKC was determined in mesangial cells grown to 80% confluence with medium FCS reduced to 0.5% for 24 hours before addition of test agents. α-Tocopherol, its dimethylsulfoxide (DMSO) vehicle (0.05%), or other test agents were added,

From the Department of Medicine, Veterans Affairs Medical Center, Pittsburgh and the University of Pittsburgh, Pittsburgh, PA.

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Address reprint requests to F.R. DeRubertis, MD, VA Medical Center, University Drive C, Pittsburgh, PA 15240.

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and culture continued for an additional 24 hours. The cells were labeled with ^{32}P-orthophosphate (New England Nuclear, Boston, MA) for 4 hours. Thromboxane or other agonists were then added, and the cells were extracted for the acid-soluble, 80-kd myristoylated alanine-rich C kinase substrate (MARCKS) protein 15 minutes later. Proteins in this extract were solubilized and separated by electrophoresis, and the PKC activity was expressed as density units determined as the ratio of phosphorylation of the 80-kd MARCKS protein to a 40-kd reference protein whose phosphorylation did not change under the various culture conditions studied. As previously reported,[5] under the experimental conditions used, the 80-kd band was immunoprecipitated by antibody to MARCKS protein. Moreover, under the current culture conditions, activation of PKC in mesangial cells by high glucose was also observed as assessed by assay of PKC activity in vitro.[25] To evaluate the effect of high glucose on PKC activity, mesangial cells were maintained in medium containing 5 or 30 mmol/L glucose and 10% FCS throughout.

Bioassay of TGFβ Activity

TGFβ bioactivity in mesangial cell culture media was determined using the mink lung epithelial cell bioassay as previously reported.[7,11] Unconditioned mesangial cell media containing the final concentration of agonists and inhibitors present in the media from mesangial cell cultures were used as controls. Total (latent + active) bioactivity was determined on samples heated to 80°C for 10 minutes and diluted to be within the detection range (0.05 to 5 pmol/L) of the assay.

Fibronectin Synthesis

Fibronectin synthesis was measured as [^{35}S]methionine (ICN, Irvine, CA) incorporated into immunoprecipitable material extracted from the cells plus matrix after a 4-hour pulse-label as previously described.[5] Synthesis is expressed as dpm/10^3 cells.

Collagen Synthesis

Collagen synthesis was determined as previously described.[26] Briefly, cells pulse-labeled with [^{3}H]proline were lysed on ice by adding acetic acid to a final concentration of 5%. After 10 minutes, the extracts were centrifuged and the cell supernatants combined with the media. Proteins were precipitated from media plus cell supernatants by addition of 33% cold ethanol and 3% bovine serum albumin. Following overnight precipitation in the cold, the pellets were washed extensively with cold 33% ethanol, dissolved in 0.5 mL 0.1-mmol/L Tris, 15 mmol/L $CaCl_2$, and 4 mmol/L *N*-ethylmaleimide and incubated for 90 minutes at 37°C in the presence or absence of 20 U/0.5 mL collagenase VII. At the end of the incubation, collagenase-insensitive proteins were precipitated with cold 20% TCA and the supernatants were counted. [^{3}H]Proline released into the supernatant of incubations conducted in the absence of collagenase was subtracted from that released by incubation with collagenase for determination of collagen synthesis.

Materials

Materials were obtained from sources already noted herein or as previously reported.[5,6,11] α-Tocopherol, taurine, and *N*-acetylcysteine (NAC) were obtained from Sigma Chemical (St Louis, MO). α-Tocopherol was diluted in DMSO and stored under nitrogen at 4°C until use.

Statistics

Experiments were performed at least three times, and the data are the mean ± SE. Each incubation condition was represented by duplicate or triplicate wells; replicates from a single culture (experiment) were averaged and entered as one value for purposes of statistical analysis. ANOVA was performed to compare multiple groups, and the significance of differences between mean values was determined by Student's *t* test.

RESULTS

Figure 1 shows collagen synthesis in mesangial cells grown in 5 versus 30 mmol/L glucose for 3 to 4 weeks and then exposed to variable concentrations of α-tocopherol for the final 72 hours of culture. [^{3}H]Proline incorporation in mesangial cells cultured in 5 mmol/L glucose was 201 ± 15 dpm/10^4 cells, a value not altered significantly by α-tocopherol at concentrations up to 100 μg/mL. Consistent with prior studies,[12,15,23] mesangial cells cultured in 30 mmol/L glucose synthesized collagen at a rate 40% greater than that maintained in 5 mmol/L glucose. α-Tocopherol (50 μg/mL) completely blocked the increases in collagen synthesis induced by 30 mmol/L glucose. Figure 2 shows that increases in fibronectin synthesis induced by 30 mmol/L glucose were also blocked by 50 μg/mL α-tocopherol. In these experiments, mesangial cells were cultured for 3 to 4 weeks in 30 mmol/L glucose, and α-tocopherol was present only during the final 72 hours of culture. As previously reported,[5] 30 mmol/L mannitol or 3-*O*-methylglucose do not stimulate fibronectin synthesis under the culture conditions used. Thus, the action of 30 mmol/L D-glucose on matrix protein synthesis was not attributable to hypertonicity per se.

Since increases in TGFβ bioactivity have been implicated in the stimulation of matrix protein synthesis in mesangial cells by glucose and other agonists, the concentration of α-tocopherol that inhibited glucose-induced increases in collagen and fibronectin synthesis was tested for the ability to alter the fibronectin synthetic response to exogenous TGFβ1 in mesangial cells grown in 5 mmol/L glucose. Figure 3 shows that the action of 50 pmol/L TGFβ1 to increase fibronectin synthesis in mesangial cells was not altered by α-tocopherol. α-Tocopherol also failed

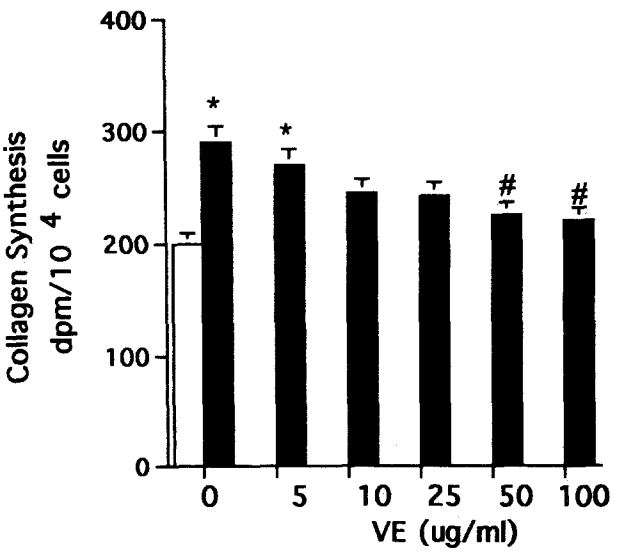

Fig 1. Effect of α-tocopherol (VE) on glucose-stimulated collagen synthesis. Mesangial cells were cultured in the presence of 5 (□) or 30 (■) mmol/L D-glucose for 3 to 4 weeks. At 72 hours before determination of collagen synthesis, the medium was changed to contain 0.5% FCS and α-tocopherol or DMSO vehicle was added. Data are expressed as [^{3}H]proline incorporation into collagenase-sensitive protein and are the mean ± SE (n = 3 to 5). Collagen synthesis in 5 mmol/L glucose was not altered by ≤100 μg/mL α-tocopherol. *$P <$ .05 *v* 5 mmol/L glucose; #$P <$ .05 *v* 30 mmol/L glucose without α-tocopherol.

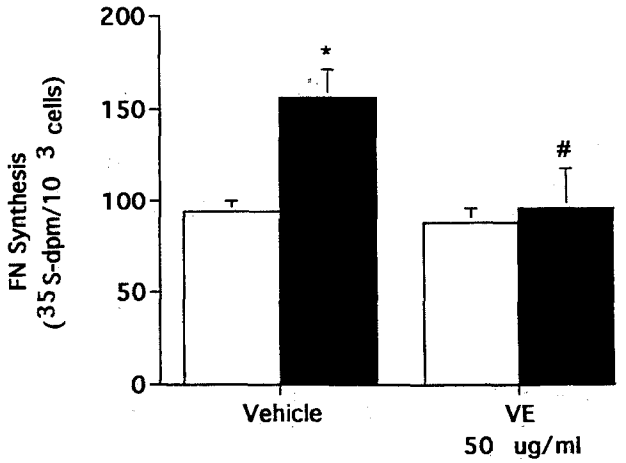

Fig 2. Effect of α-tocopherol on glucose-stimulated fibronectin (FN) synthesis. Mesangial cells were treated as described in Fig 1, and FN synthesis was assayed. α-Tocopherol was present in the culture medium for the final 72 hours of culture. Values are the mean ± SE (n = 3). (□) 5 μmol/L glucose; (■) 30 mmol/L glucose. *$P < .05$ *v* 5 mmol/L glucose; #$P < .05$ *v* corresponding values without α-tocopherol.

to suppress increases in fibronectin synthesis induced by lower (1 to 25 pmol/L) concentrations of exogenous TGFβ1 (data not shown).

Figure 4 shows TGFβ bioactivity in the media of mesangial cells exposed to high glucose concentrations for 3 to 4 weeks with or without addition of 50 μg/mL α-tocopherol for the final 72 hours of culture. The media concentration of active TGFβ was threefold higher in mesangial cells cultured in 30 versus 5 mmol/L glucose, whereas total TGFβ was twofold higher. α-Tocopherol did not alter TGFβ in media of mesangial cells cultured with 5 mmol/L glucose. However, the presence of α-tocopherol suppressed the media content of active and total

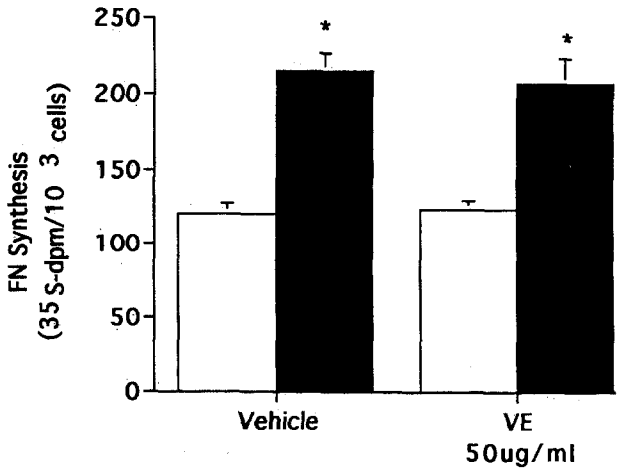

Fig 3. Effect of α-tocopherol on TGFβ-stimulated fibronectin (FN) synthesis. Mesangial cells were cultured in 5 mmol/L glucose as described in Fig 1. 50 μg/mL α-tocopherol or DMSO vehicle was added to the cultures 24 hours before addition of 50 pmol/L exogenous TGFβ1, and FN synthesis was determined 24 hours later. Values are the mean ± SE (n = 4 to 6). (□) Control; (■) TGFβ (50 pmol/L). *$P < .05$ *v* control.

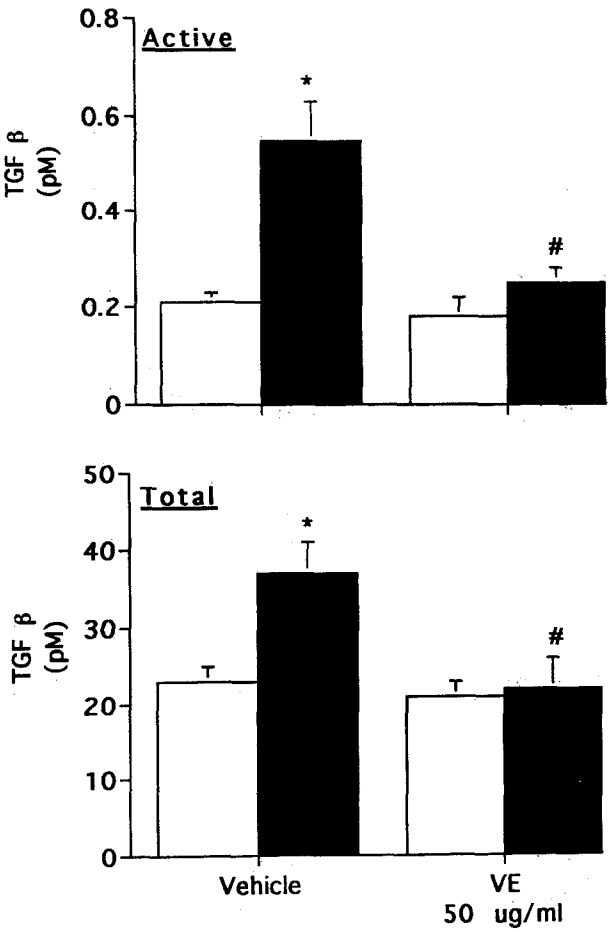

Fig 4. Effect of α-tocopherol on glucose-stimulated TGFβ bioactivity. Mesangial cells were treated as described in Fig 1. The cells were cultured in media containing 10% FCS with or without 50 μg/mL α-tocopherol for 48 hours. This medium was replaced with fresh medium containing 0.5% FCS and 5 (□) or 30 (■) mmol/L glucose with or without α-tocopherol. The low serum media was then harvested for assay of TGFβ bioactivity after an additional 24 hours of culture. Values are the mean ± SE (n = 4 to 6) and are normalized to 10^5 cells. *$P < .05$ *v* 5 mmol/L glucose; #$P < .05$ *v* corresponding value without VE.

TGFβ in mesangial cells cultured in 30 mmol/L glucose to values no different from those observed in 5 mmol/L glucose.

Figure 5 shows the effect of α-tocopherol on glucose-stimulated PKC activity in mesangial cells. Compared with values in 5 mmol/L glucose, PKC activity was 65% higher in mesangial cells exposed to 30 mmol/L glucose for 24 hours; addition of 50 μg/mL α-tocopherol to the cultures concurrent with increasing media glucose from 5 to 30 mmol/L prevented the increase in PKC induced by high glucose. Furthermore, in mesangial cells cultured with high glucose for 7 to 12 days, the presence of α-tocopherol for the final 72 hours of culture suppressed PKC activity to values not different from those in mesangial cells cultured in 5 mmol/L glucose.

N-Acetylcysteine enhances cellular capacity to buffer reactive oxygen moieties by increasing intracellular glutathione.[27] Figure 6 shows the effect of *N*-acetylcysteine on PKC activity and TGFβ bioactivity in mesangial cells cultured with 5 or 30 mmol/L glucose for 7 to 12 days. *N*-acetylcysteine had no effect

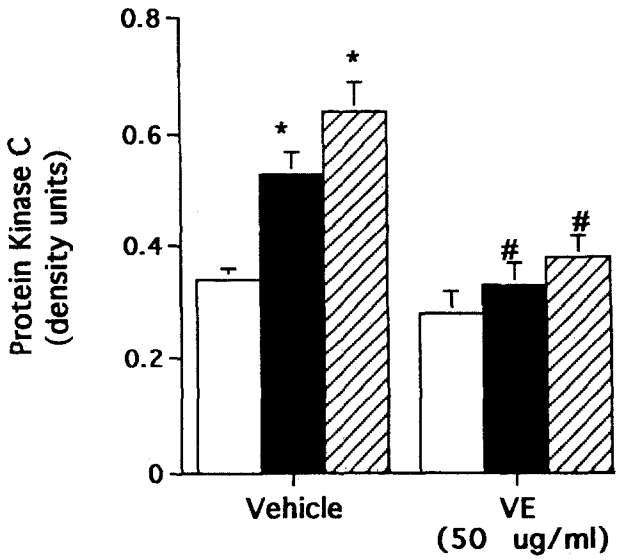

Fig 5. Effect of α-tocopherol on glucose-stimulated PKC activity. In the 24-hour experiments shown, mesangial cells were maintained in MEM containing 5 mmol/L (□) glucose. Media glucose was increased to 30 mmol/L (■), and 50 μg/mL α-tocopherol was added concurrently, where indicated, 24 hours before determination of MARCKS protein phosphorylation. In other studies, mesangial cells were cultured with 30 mmol/L glucose for 7 to 12 days (▨), and α-tocopherol was present for the final 72 hours before PKC assay. Values are the ratio of densities of the 80-kd MARCKS protein and a 40-kd reference band and are the mean ± SE (n = 3 to 6). *$P < .05$ *v* 5 mmol/L glucose; #$P < .05$ *v* corresponding value without α-tocopherol.

on MARCKS protein phosphorylation in mesangial cells grown in 5 mmol/L glucose, but when present for the final 24 hours of culture, it completely blocked the twofold increase in PKC activity induced by 30 mmol/L glucose. *N*-Acetylcysteine also prevented increases in TGFβ bioactivity induced by high glucose. Taurine is an amino acid with antioxidant properties.[28] Figure 7 shows that the presence of taurine for the final 72 hours of culture blocked the increases in PKC activity and media TGFβ bioactivity induced by culture of mesangial cells with 30 mmol/L glucose for 7 to 12 days.

The effects of α-tocopherol on mesangial cell responses to thromboxane are shown in Fig 8. In these experiments, cells were cultured with 5 mmol/L glucose and α-tocopherol was added to the cultures 24 hours before thromboxane. α-Tocopherol, 5 μg/mL, significantly suppressed thromboxane-induced increases in fibronectin synthesis, and α-tocopherol, 10 to 25 μg/mL, completely blocked this thromboxane response. At these concentrations, α-tocopherol also suppressed or completely blocked the increases in TGFβ bioactivity and PKC induced by thromboxane in mesangial cells.

Figure 9 compares the effects of α-tocopherol and those of two other antioxidants on the activation of PKC in mesangial cells by thromboxane, AII, and phorbol dibutyrate (PDBu). α-Tocopherol blocked PKC activation induced by thromboxane and AII. It suppressed but did not abolish activation of PKC in mesangial cells by 0.1 μmol/L PDBu, which induced maximal increases in PKC (Fig 9), and by submaximal (10 and 50 nmol/L) concentrations of PDBu (data not shown). However, α-tocopherol did not alter the PKC response of mesangial cells when a supramaximal concentration of PDBu (0.5 μmol/L) was used (0.5 μmol/L PDBu, 1.75 ± 0.05; PDBu ± α-tocopherol, 1.95 + 0.11; n = 5). *N*-Acetylcysteine and taurine each blocked activation of PKC by thromboxane or AII. However, neither *N*-acetylcysteine nor taurine altered the effects of PDBu on PKC.

DISCUSSION

Stimulation of matrix protein synthesis in glomerular mesangial cells by high glucose, thromboxane, AII, and other agonists involves activation of PKC and increases in TGFβ.[4-8,14] Previous studies have demonstrated that α-tocopherol blocks activation of PKC induced by high glucose in several cell types,[19-21] and that α-tocopherol suppresses increases in collagen synthesis

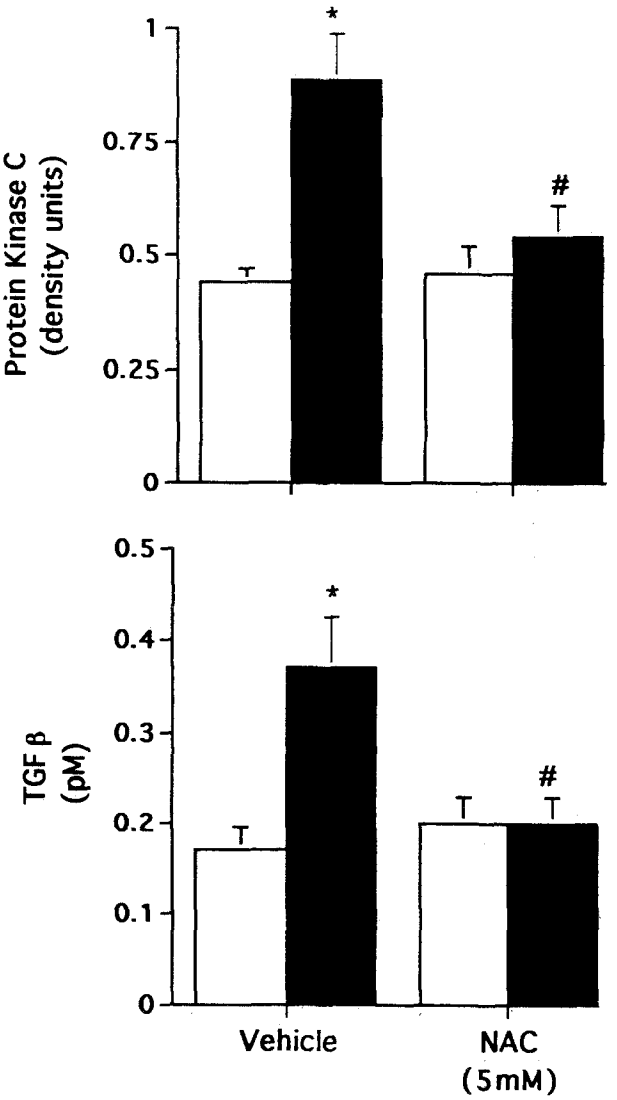

Fig 6. Effect of *N*-acetylcysteine on increases in PKC activity and TGFβ induced by culture of mesangial cells with high glucose. Mesangial cells were cultured in media containing 5 (□) or 30 (■) mmol/L glucose for 7 to 12 days; 5 mmol/L *N*-acetylcysteine was present for the final 24 hours of culture where indicated. PKC activity and TGFβ bioactivity were determined. Values are the mean ± SE (n = 3 to 5). *$P < .05$ *v* 5 mmol/L glucose; #$P < .05$ *v* corresponding value without *N*-acetylcysteine.

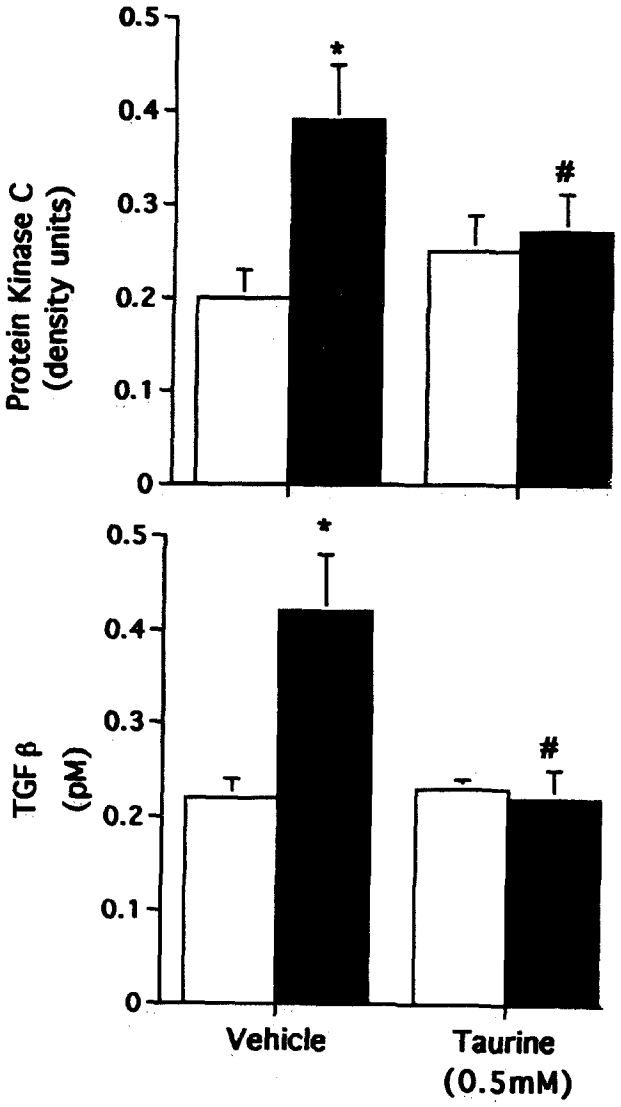

Fig 7. Effect of taurine on glucose-stimulated TGFβ bioactivity and PKC activity. Mesangial cells were cultured for 7 to 12 days as described in Fig 1; 0.5 mmol/L taurine was present for the final 72 hours of culture where indicated. PKC activity and TGFβ bioactivity were determined. Values are the mean ± SE (n = 3 to 5). (□) 5 mmol/L glucose; (■) 30 mmol/L glucose. *$P < .05$ *v* 5 mmol/L glucose; #$P < .05$ *v* corresponding value without taurine.

induced by high glucose in mesangial cells.[23] The present study provides evidence that the action of α-tocopherol and other antioxidants to suppress stimulation of matrix protein synthesis by high glucose in mesangial cells is linked to their capacity to block PKC-signaled increases in TGFβ induced by high glucose. Thus, α-tocopherol blocked activation of PKC, increases in TGFβ, and matrix protein synthesis induced by high glucose, but did not alter the increases in matrix protein synthesis induced by exogenous TGFβ1. The capacity of α-tocopherol and other antioxidants to prevent increases in TGFβ induced by high glucose in mesangial cells has not been documented previously. In this regard, analogous effects are observed with selective inhibitors of PKC and prior downregulation of PKC in mesangial cells. These PKC inhibitors or PKC depletion also fail to alter stimulation of matrix protein synthesis by exogenous TGFβ1, but prevent increases in PKC activity, endogenous TGFβ bioactivity, and matrix protein synthesis induced by high glucose, thromboxane, low-density lipoproteins, and other PKC agonists.[7,10-12] By contrast, anti-TGFβ antibody blocks stimulation of matrix protein synthesis by these same agents in mesangial cells without altering activation of PKC.[7,10-12] Moreover, exogenous TGFβ does not activate PKC

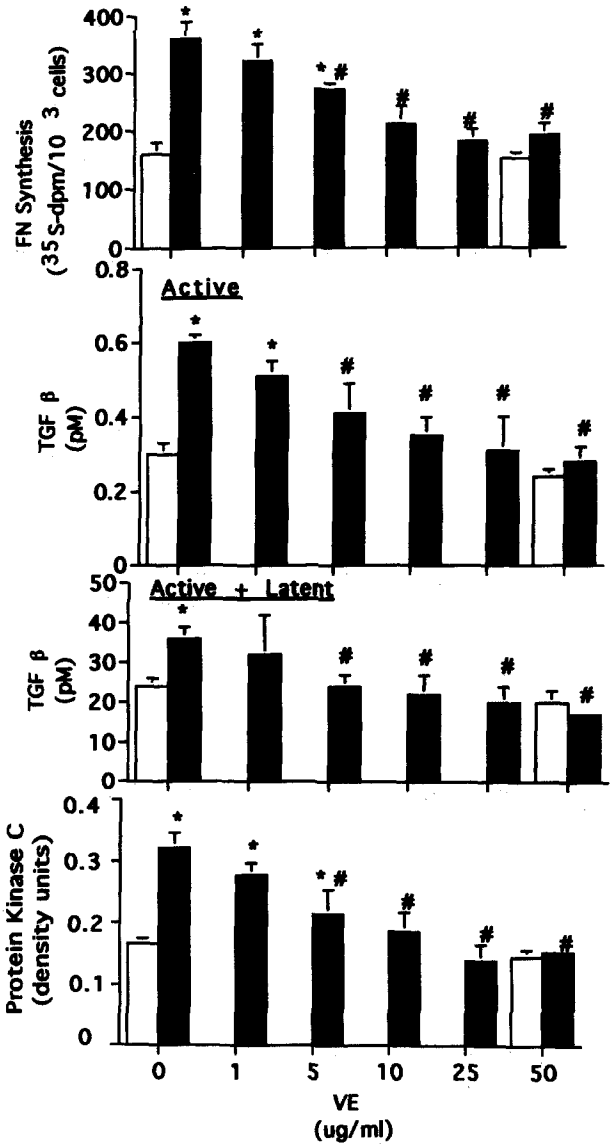

Fig 8. Effect of α-tocopherol on U46619-stimualted fibronectin synthesis, TGFβ bioactivity, and PKC activity. Mesangial cells were grown to confluence in medium containing 5 mmol/L glucose and 10% FCS. Medium FCS was then reduced to 0.5% and 50 μg/mL α-tocopherol or the DMSO vehicle was added as indicated. After 24 hours of culture, fresh medium with or without 1 μmol/L U46619 was introduced. Where indicated, mesangial cells were harvested for determination of MARCKS protein phosphorylation 15 minutes after addition of U46619. Other cultures were harvested 24 hours after addition of U46619 for determination of media TGFβ content or fibronectin (FN) synthesis. (□) Control; (■) 1 μmol/L U46619. Values are the mean ± SE (n = 4 to 6). *$P < .05$, U46619 *v* control; #$P < .05$, U46619 + α-tocopherol *v* U46619 alone.

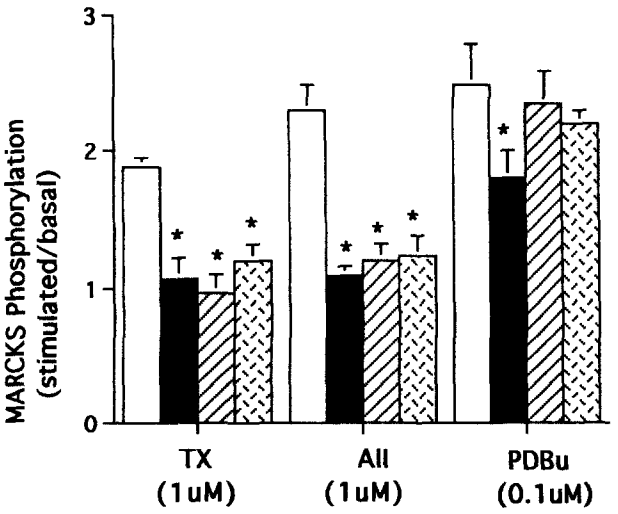

Fig 9. Effects of α-tocopherol, *N*-acetylcysteine, and taurine on activation of PKC by U46619, AII, and PDBu. Mesangial cells were cultured as described for PKC in Fig 8 and cultured with no antioxidant (□), α-tocopherol (■), *N*-acetylcysteine (▨), or taurine (▩) for 24 hours before addition of thromboxane (TX), AII, or PDBu; MARCKS protein phosphorylation was determined 15 minutes after addition of these PKC agonists. Data are expressed as the ratio of agonist-stimulated MARCKS phosphorylation to basal levels of phosphorylation in the same experiments. Values are the mean ± SE (n = 3 to 6). *$P < .05$ *v* corresponding value in the absence of an antioxidant.

in mesangial cells.[11] Taken together, these findings suggest that α-tocopherol blocks the stimulatory effects of high glucose on matrix protein synthesis in mesangial cells by interfering with PKC-signaled increases in endogenous TGFβ. Similarly, two other agents with antioxidant activity, *N*-acetylcysteine and taurine, prevent activation of PKC and increases in TGFβ bioactivity induced in mesangial cells by high glucose. These results suggest that activation of PKC by high glucose may involve an oxidant mechanism that is amenable to suppression by structurally distinct aqueous and lipid-phase antioxidant agents. Oxidative activation of PKC has been demonstrated in cell homogenates,[17] and reactive oxygen species have been linked to PKC activation in hepatocytes.[16] Similarly, reactive oxygen species may be involved in the activation of PKC by AII.[22]

Of note, α-tocopherol, taurine, and *N*-acetylcysteine not only block the PKC response of mesangial cells to high glucose, but also suppress the receptor-mediated activation of PKC in response to thromboxane and AII and the PKC-signaled increases in TGFβ and matrix protein synthesis induced by thromboxane. The latter actions of these antioxidants have not been previously reported and have implications with respect to the potential mechanisms by which they suppress PKC activation. Thus, activation of PKC by high glucose in mesangial cells and other cells has previously been linked to increases in the endogenous cellular PKC agonist diacylglycerol (DAG) formed via de novo lipid synthesis.[3] Although not specifically measured in the present study, inhibition of glucose-induced DAG accumulation by α-tocopherol has been demonstrated in VSMCs, retinal endothelial cells, and isolated glomeruli.[19-21] Probucol, a lipid-lowering agent with antioxidant properties, similarly has been shown to prevent increases in DAG induced by high glucose in VSMCs.[19] In contrast to high glucose, thromboxane and AII enhance cellular DAG accumulation and activate PKC via receptor-mediated membrane phospholipid hydrolysis. The mechanisms by which structurally diverse antioxidants alter PKC activation in response to agonists that increase DAG generation by distinct metabolic pathways remain to be determined. It is possible that antioxidants interfere with DAG formation via both de novo synthesis from glucose and membrane phospholipid hydrolysis. Alternatively, antioxidants might suppress PKC activation by interfering with the interaction of DAG with PKC through a direct effect on this enzyme system that is expressed independently of the cellular metabolic pathway by which DAG is generated. The current studies support the possibility that the inhibitory effect of α-tocopherol on PKC activation in mesangial cells is expressed, at least in part, via this mechanism. Thus, α-tocopherol partially blocked PKC activation by submaximal and maximal doses of the DAG analog PDBu. This inhibitory effect of α-tocopherol on PKC was overcome by a supramaximal concentration of PDBu, consistent with a competitive interaction of PDBu and α-tocopherol with PKC. Similar results were reported by Kochs et al,[29] who found that α-tocopherol acted as a weak competitive inhibitor of DAG binding to a purified preparation of PKCα. Boscoboinik et al[30-32] also observed that α-tocopherol blocked PDBu activation of PKC in cultured VSMCs during some but not all phases of the growth cycle of these cells. By contrast, Kunisaki et al[19] did not observe α-tocopherol inhibition of PDBu-responsive PKC activity of subcellular fractions of VSMCs. Although α-tocopherol appears to act as a competitive inhibitor of PKC activation by PDBu in mesangial cells and thus may interfere with DAG action on the enzyme, neither *N*-acetylcysteine nor taurine altered PDBu-responsive PKC activity when tested at concentrations that clearly blocked PKC responses to high glucose, thromboxane, and AII. Thus, direct effects of these antioxidants on the interaction of DAG with PKC seem unlikely. An additional mechanism at the level of DAG degradation could account for antioxidant inhibition of PKC activation in response to agents that increase DAG generation via different pathways. In this regard, α-tocopherol has been reported to increase DAG kinase activity in endothelial cells.[33] Preliminary findings in VSMCs[34] and in glomeruli[35] isolated from diabetic rats also support this mechanism. Probucol similarly was found to increase DAG kinase activity in VSMCs and to inhibit PKC activation by glucose,[34] findings consistent with an antioxidant effect on DAG degradation. Figure 10 is a schematic representation of the proposed cascade of events leading to stimulation of MC matrix expansion and the potential sites of antioxidant action as supported by the current and previous studies. NAC and taurine block activation of PKC by both receptor-mediated DAG formation via hydrolysis of membrane phospholipid and de novo synthesis of DAG from glucose, but do not block PKC activation by phorbol ester. These results, combined with other recent reports,[33-35] are consistent with an antioxidant effect to inhibit DAG accumulation. By contrast, α-tocopherol not only blocked activation of PKC by agents that increase endogenous DAG levels, but also suppressed phorbol ester activation of PKC. Thus, in addition to

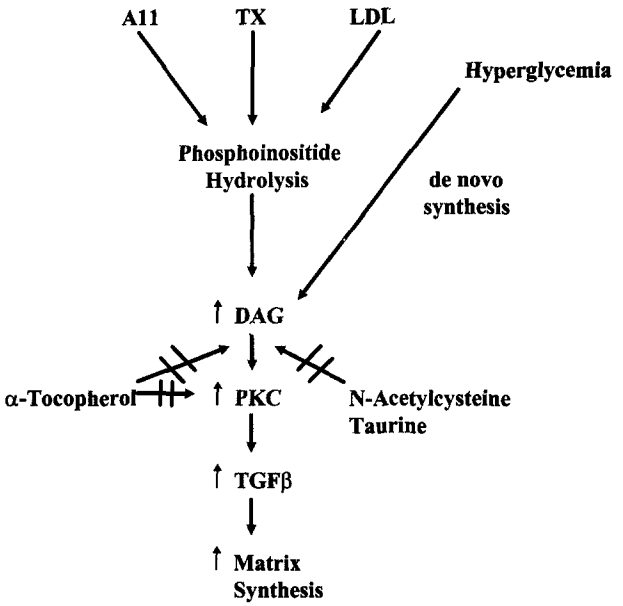

Fig 10. Schematic representation of potential sites of antioxidant interference in mesangial cell matrix protein synthesis.

its reported action to enhance DAG degradation,[35] α-tocopherol may also have direct inhibitory effects on PKC.[29]

The current study is the first to demonstrate that antioxidants inhibit increases in TGFβ bioactivity in response to high glucose or thromboxane in mesangial cells, presumably, at least in part, by interfering with the PKC signal. There is now considerable evidence that TGFβ is involved in the pathogenesis of glomerulosclerosis in diabetic and nondiabetic nephropathies.[13,14] The capacity of antioxidants to prevent increases in TGFβ and TGFβ-dependent matrix protein synthesis in mesangial cells suggests both the potential utility of these agents in attenuating glomerular injury and a possible mechanism for a therapeutic effect. In this regard, Trachtman et al[36] have recently demonstrated that treatment with taurine retards the decline in glomerular filtration rate, the increases in urinary albumin excretion, and the development of glomerulosclerosis in diabetic rats. Similarly, taurine has been reported to attenuate glomerulosclerosis in puromycin-induced nephropathy.[37] By contrast, treatment with low-dose α-tocopherol (threefold > control intake) appeared to accelerate the deterioration of renal function and the development of glomerulosclerosis and to increase mortality in diabetic rats.[36] The latter may have been due to conversion of low-dose α-tocopherol to a prooxidant[38] in the setting of increased oxidative stress induced by the marked hyperglycemia present in the diabetic model used.[36] In studies of shorter duration, higher doses of α-tocopherol have been reported to attenuate glomerular hyperfiltration.[21] Thus, although there are data to suggest beneficial effects of antioxidant agents on the renal changes induced by diabetes, further studies are needed to assess their potential therapeutic utility and the mechanisms by which they may alter glomerular injury.

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