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Article

Angiotensin II induces human TGF-β1 promoter activation: similarity to hyperglycaemia

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Abstract

Aims/hypothesis. Activation of the renal renin-angiotensin system has been implicated in the pathogenesis of diabetic nephropathy. Because previous in vitro studies demonstrated the angiotensin II (ang II)-mediated up-regulation of the prosclerotic transforming growth factor β1 (TGF) we studied the molecular mechanism of ang II-induced TGF-β1 gene activation.
Methods. Mesangial cells were stimulated with 100 nmol/l ang II with or without inhibitors of protein kinase C (PKC) and p38 MAPK and the TGF-β1 promoter activity was determined by promoter-reporter assays. The effect of ang II on the binding of nuclear proteins to the regulatory AP-1 site B, previously shown to mediate the high glucose-response of the TGF-β1 promoter, was studied by electrophoretic mobility shift assays.
Results. Ang II enhanced the activity of the TGF-β1 promoter fragment –453/+11 approximately 1.6-fold. Mutation of each of two AP-1 binding sites or inhibition of the PKC- and p38 MAPK-dependent pathways blocked the ang II-stimulated activity completely. Furthermore, ang II activated the binding of nuclear proteins to the AP-1 box B of the TGF-β1 promoter. These effects were similar to those previously observed with high glucose. Co-incubation with ang II and high glucose had no additive effect on TGF-β1 promoter activity, protein binding to the AP-1 box B or activation of p38 MAPK.
Conclusion/interpretation. The findings indicate that ang II and hyperglycaemia stimulate the TGF-β1 gene activation through the same PKC- and p38 MAPK-dependent pathways by the same regulatory elements of the TGF-β1 promoter. Our data could also be relevant for e.g. hypertension-induced glomerulosclerosis. [Diabetologia (2002) 45:890–898]

Keywords Diabetic nephropathy, gene expression, protein kinase C, p38 MAPK, transcription factor AP-1.

Studies have provided evidence that angiotensin-converting enzyme inhibitors and angiotensin-receptor antagonists attenuate diabetic glomerulosclerosis and slow the progression of diabetic kidney disease [1, 2, 3, 4, 5, 6, 7]. It has been suggested that these effects are, at least in part, independent from the reduction of the systemic blood pressure [8, 9, 10]. These results indicate that angiotensin II (ang II) could have direct effects on glomerular, particularly mesangial cells by inducing sclerosis independent of its haemodynamic actions [11, 12].

The prosclerotic cytokine transforming growth factor beta (TGF-β1) has been implicated as an important downstream mediator in the progression of the renal pathological changes occurring in diabetic patients which lead to glomerular and tubular basement membrane thickening, mesangial matrix expansion, and glomerulosclerosis [13, 15, 15, 16, 17, 18]. Numerous studies including data from our own laboratory indi-

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Abbreviations: Ang II, Angiotensin II; EMSA, electrophoretic mobility shift assay; MAPK, mitogen-activated protein kinase; PKC, protein kinase C; PMA, phorbol myristate acetate

cate that hyperglycemia induces an increase in TGF-β1 protein and mRNA expression in experimental and human diabetes [15, 19, 20, 21] and in cultured mesangial cells [22, 23, 24]. Although previous investigations clearly demonstrated that ang II stimulates the synthesis of extracellular matrix proteins through an enhanced TGF-β1 expression in mesangial cells [25] the molecular mechanism of ang II-induced TGF-β1 gene activation, particularly the detailed localization of the ang II responsive elements on the TGF-β1 promoter, have not been studied yet.

Studies with cultured cells, e.g. smooth muscle cells and renal tubular epithelial cells, indicate an activation of protein kinase C and p38 MAPK by ang II [11, 26, 27, 28]. Furthermore, other reports have shown that ang II induces the DNA-binding activity of AP-1 proteins [29, 30, 31, 32]. These observations suggest the possibility of an involvement of PKC, p38 MAPK and AP-1 in mediating non-haemodynamic effects of ang II.

The human TGF-β1 promoter region contains two AP-1 binding sequences [33], designated box A and B [34], which mediate the up-regulation of promoter activity through an PKC-dependent pathway after treatment of cells with phorbol esters [35] or after stimulation with high glucose concentrations [34]. In a recent study from our laboratory we could show that the high glucose-enhanced TGF-β1 gene activation is mediated through PKC- and p38 MAPK-dependent pathways and that it involves enhanced binding of AP-1 proteins predominantly to the AP-1 box B in the TGF-β1 promoter, which is responsible for TGF-β1 up-regulation [34].

Based on these results, we hypothesised that ang II could increase TGF-β1 gene expression by enhancing the TGF-β1 promoter activity through the AP-1 binding sites in mesangial cells. To address this question we studied the effect of ang II on TGF-β1 gene activation using a construct of the human TGF-β1 promoter. Furthermore, the effect of ang II on the binding of AP-1 proteins to the respective DNA sites in the TGF-β1 promoter was studied by electrophoretic mobility shift assay (EMSA). Because high glucose also activates TGF-β1 gene expression, we investigated if hyperglycaemia and ang II stimulate TGF-β1 gene activation through the same or different pathway.

Subjects and methods

Materials. Oligonucleotides were synthesised by Life Technologies (Karlsruhe, Germany). Cell culture media, supplements, Ultroser and fetal calf serum (FCS) were purchased from Gibco (Eggenstein, Germany); minocyclin was from Pan Systems (Aidenbach, Germany); Superfect was from Qiagen (Hilden, Germany); β-galactosidase assay chemiluminescent, Klenow enzyme and poly[d(I-C)] were from Boehringer (Mannheim, Germany); pSV-β-galactosidase control vector (pSVβ-Gal), and luciferase assay reagent were from Promega (Madison, USA); protein assay reagent was from Bio-Rad (Munich, Germany); ang II, saralasin, PD 123319, bisindolylmaleimide I were from Sigma (Munich, Germany); SB 203580 was from Calbiochem (Schwalbach, Germany), [α-^{32}P]dATP was from Hartmann (Braunschweig, Germany). The TGF-β1-luciferase reporter vector pGL3/453 and the mutated vectors Amut, Bmut, ABmut and Sp1mut as depicted in Fig. 1 were prepared and characterised as recently described [34]. Losartan was kindly provided by Merck, Sharp and Dohme (Cramlington, Great Britain).

Cell culture. Mesangial cells isolated from porcine glomeruli were cultured and characterised as described previously [23]. Cells were grown in RPMI 1640 with 15% fetal calf serum, 1 mmol/l sodium pyruvate, 3 mmol/l glutamine, nonessential amino acids, 100 U/ml penicillin, 100 µg/ml streptomycin, 0.5 µg/ml minocyclin and 10 mmol/l glucose. For experiments RPMI 1640 containing 6 mmol/l glucose was used and fetal calf serum was substituted by 2% Ultroser. For equal osmolarity in the high glucose experiments NaCl was added to control cells.

Transfection methods. The mesangial cells were transfected with Superfect according to the instructions of the supplier. One day before transfection 1.5×10^5 cells/well were seeded in 6-well plates with 2 ml growth medium. For each well, 2 µg (1.75 µg pGl3bTGF-β1 promoter construct, 0.25 µg pSVβ-Gal) total DNA, 100 µl RPMI 1640 without supplements were premixed, 10 µl Superfect was added and the samples were mixed for 10 s. After 10 min the samples were diluted with 600 µl growth medium and the total volume was transferred to the cells. After 3 h the transfection media were replaced by different experimental media (100 nmol/l ang II, 30 mM glucose, 100 nmol/l ang II and 30 mmol/l glucose) with or without the different antagonists or inhibitors and cells were harvested after 24 h.

Reporter gene assays. Transfected cells were washed once with PBS, incubated with 150 µl lysis buffer from the β-galactosidase chemiluminescent assay for 30 min and harvested. After 2 min centrifugation the supernatants were stored at –80°C or immediately used for the measurements. β-Galactosidase activities and firefly luciferase activities were determined according to the instructions of the manufactures. Chemiluminescence was determined with a Magic Lite Analyzer from Ciba Corning (Fernwald, Germany). Luciferase activities were normalised to β-galactosidase activities. All transfection experiments were repeated at least three times.

Preparation of nuclear extracts. For preparation of nuclear extracts 6.0×10^6 cells were seeded onto 15-cm culture dishes and cultured in 15 ml experimental medium. Cells were incubated with 100 nmol/l ang II containing 6 or 30 mmol/l glucose or 30 mmol/l glucose alone for 24 h before harvesting. Nuclear proteins were prepared as described by Andrews and Faller [36] and the protein concentrations were determined according to Bradford using the Bio-Rad protein assay reagent [37].

Electrophoretic mobility shift assay. Appropriate oligonucleotides were endlabelled with [α-^{32}P]dATP (3000 Ci/mmol/l) and Klenow enzyme and were incubated with up to 10 µg nuclear protein in 20 µl 7 mmol/l Hepes-KOH pH 7.9, 100 mmol/l KCl, 3.6 mmol/l $MgCl_2$, 10% glycerol on ice for 20 min. 0.05 mg/ml poly[d(I-C)] was added as unspecific competitor. The samples were run on a 5% non-denaturating polyacrylamide gel in a buffer containing 25 mmol/l Tris-HCl pH 8.0; 190 mmol/l glycine and 1 mmol/l EDTA. Gels were dried and

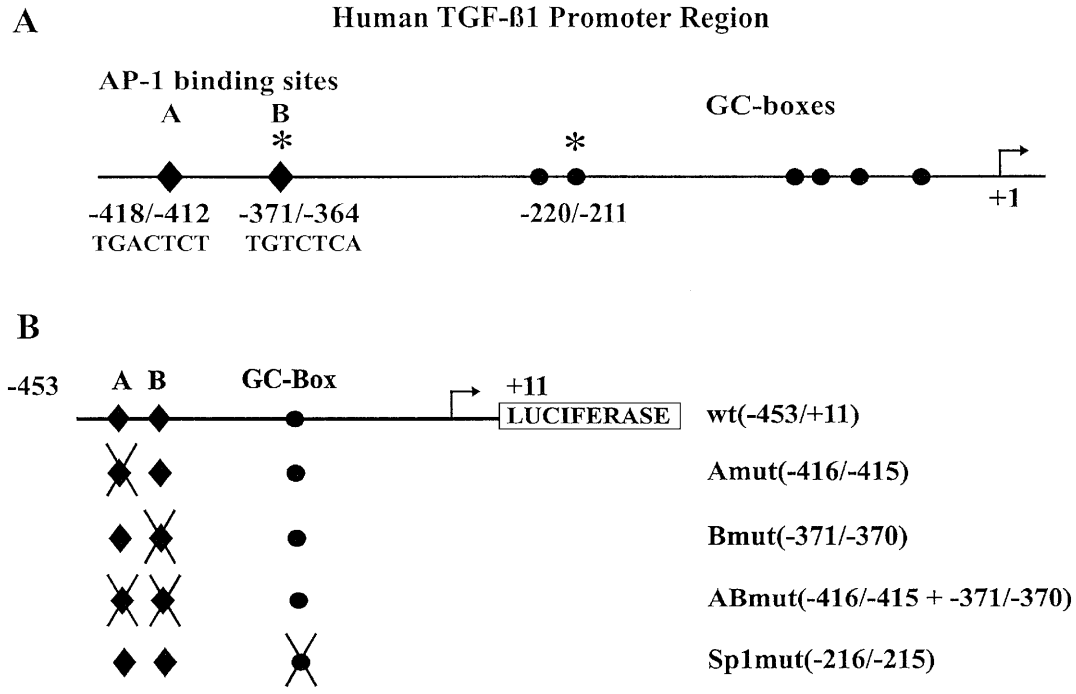

Fig. 1A, B. A Cis-regulatory elements in the 5'-flanking region of the human TGF-β1 gene. Positions are deduced from sequence data as described previously [33]. The AP-1-like binding sites are indicated by ◆, ● show GC-boxes. The AP-1-like sites A and B mediate the high glucose-response and phorbol ester-response of the promoter and are also necessary for basal promoter function [34]. Binding sites investigated by electrophoretic mobility shift assays are indicated by an *asterisk*. **B** Constructs of the 5′-flanking region of the TGF-β1 gene in pGL3basic which contains the firefly (photinus pyralis) luciferase coding region. The pGL3/453 includes the wild-type TGF-β1 fragment –453/+11 fused to the luciferase gene. The AP-1 binding sites A in Amut, B in Bmut, A and B in ABmut and the Sp1 binding site in Sp1mut were mutated by a change of two bases as indicated

analysed by autoradiography. The synthetic oligonucleotides used in electrophoretic mobility shift assays correspond to base pairs –385/–351 (GGTCGGCTCCCCTGTGTCTCATCCCCCGGATTAAC) and –229/–196 (AGCCGGGGAGCCCGCCCCCTTTCCCCCAGGGCTG) and cover the AP-1 B and Sp-1 binding sites as shown in Fig. 1.

Western blots. Nuclear extracts of mesangial cells were separated by sodium dodecyl sulfate polyacrylamide (7.5%) gel electrophoresis. Proteins were transferred to nitrocellulose by semi-dry-electroblotting (transfer buffer: 48 mmol/l Tris, 39 mmol/l glycine, 0.0375% sodium dodecyl sulfate, 20% (v/v) methanol). Then nitrocellulose membranes were blocked with NET buffer (150 mmol/l NaCl, 50 mmol/l Tris/HCl pH 7.4, 5 mmol/l EDTA, 0.05% Triton X-100, 0.25% gelatine) and incubated with the first antibody (diluted 1:1000 in NET) overnight at 4°C. After washing with NET the membranes were incubated with horseradish peroxidase-conjugated anti-rabbit or anti-goat IgG for 1 h at room temperature. Visualisation of immunocomplexes was performed by enhanced chemiluminescence.

Statistical analysis. Results presented are derived from at least three independent experiments. Means ± SEM were calculated and groups of data were compared using Student's *t* test; if necessary Dunnett's correction for multiple comparisons was performed. A *p* value of less than 0.05 was considered to be statistically significant.

Results

Ang II increased the activity of the human TGF-β1 promoter. Transfection experiments revealed an ang II-induced stimulation of the human TGF-β1 promoter fragment –453/+11 (schematically depicted in Fig. 1) as assessed by luciferase activity. Addition of ang II for 24 h in the presence of normoglycaemia (6 mmol/l glucose) stimulated the promoter activity significantly (1.6-fold) when compared with control cells (Fig. 2). The extend of this activation was similar to the recently demonstrated TGF-β1 promoter activation by high glucose concentrations [34]. Addition of the competitive ang II-receptor antagonist saralasin or the ang II-receptor type 1 (AT1)-specific antagonist losartan prevented the up-regulation completely while basal activity was not affected (Fig. 2), whereas in the presence of the ang II-receptor type 2 (AT2)-specific antagonist PD 123319 the ang II-induced promoter activation was not prevented (Fig. 2). These data indicate that the TGF-β1 promoter activation by ang II is mediated through the AT1-receptor.

Localisation of the responsible cis-regulatory elements. To study whether the two AP-1 binding sites –418/–412 and –371/–364 mediate the ang II effect, we transfected the mesangial cells with the mutated constructs depicted in Fig. 1. As shown in Fig. 3 mu-

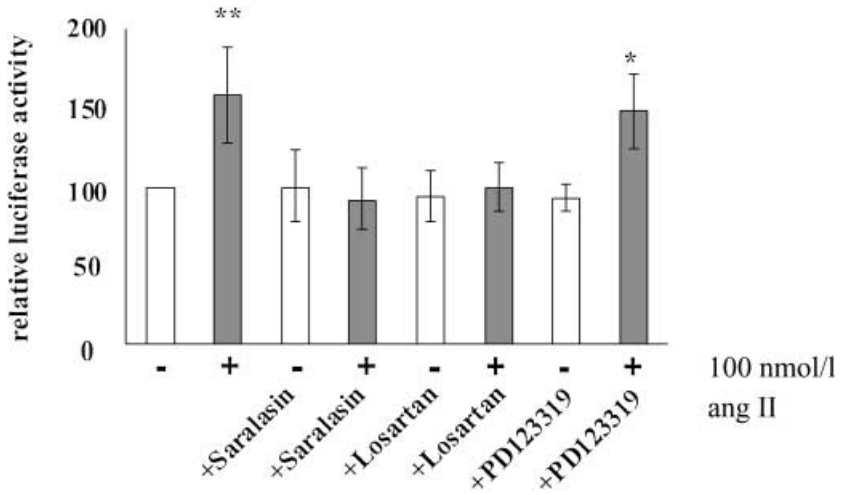

Fig. 2. Effect of ang II on the TGF-β1 promoter activity. Mesangial cells were transfected with the wild-type plasmid pGL3/453 and cultured in 6 mmol/l glucose with or without 100 nmol/l ang II for 24 h. 10 µmol/l Saralasin, 10 µmol/l losartan or 10 µmol/l PD 123319 were added simultaneously with ang II as indicated. Luciferase activities are expressed as percentages of the activity measured with 6 mmol/l glucose. Three different independent experiments were performed. Results shown are means ± SEM. *p<0.05 ang II vs control; **p<0.01 ang II vs control

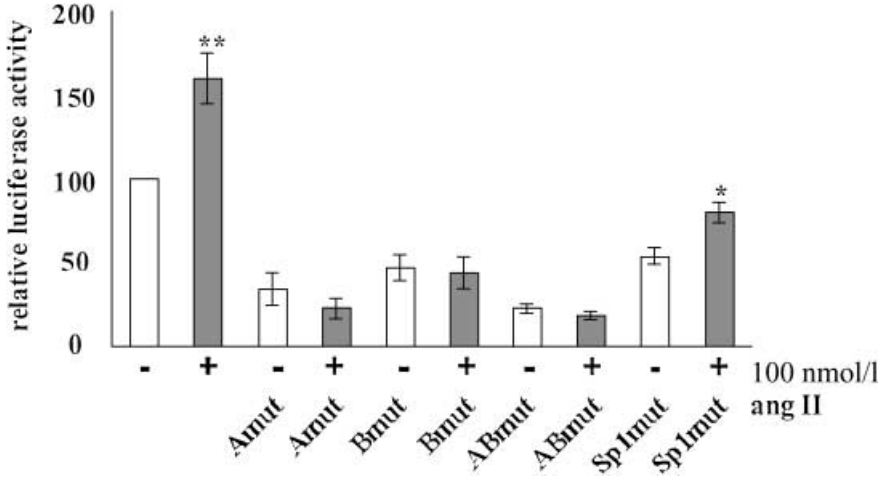

Fig. 3. Effect of base mutations in the TGF-β1 promoter region on the ang II-induced promoter activity. The wild-type and the mutated plasmids used for the transfection experiments are shown in Fig. 1B. Mesangial cells were transfected with equal amounts of the different plasmids and grown in 6 mmol/l glucose with or without 100 nmol/l ang II for 24 h as indicated. Luciferase activities are expressed as percentages of the activity measured with the wild-type plasmid and 6 mmol/l glucose. Data are means ± SEM of three different experiments. *p<0.05 ang II vs control (Sp1mut); **p<0.01 ang II vs control

tation in AP-1 box A or box B or mutation in both boxes completely prevented the ang II-induced increase of the TGF-β1 promoter activity and reduced the promoter activity below control levels. The lowest remaining promoter activity was found after mutation of both AP-1 sites (about 25%), whereas mutation of AP-1 box B had the lowest effect on basal promoter activity (approximately 50%). Furthermore, we examined the function of an high-affinity Sp1 binding site (–220/–211). Although mutation of this GC-box (see Fig. 1) reduced both the basal and the ang II-stimulated promoter activity the relative ang II-induced stimulation remained significant (1.6-fold) (Fig. 3). These data identifying the two AP-1 binding sites in the human TGF-β1 promoter fragment –453/+11 as the ang II-responsive elements correspond to the results obtained with high glucose [34] suggesting that both stimuli could activate TGF-β1 gene expression by the same molecular mechanism. Comparison of ang II- and high glucose-induced TGF-β1 promoter activities are shown in Table 1.

Ang II-induced promoter activation is mediated through PKC- and p38 MAPK-dependent pathways with no further stimulation by high glucose. Therefore we evaluated whether co-stimulation of the mesangial cells with 100 nmol/l ang II and 30 mmol/l glucose further increased the TGF-β1 promoter activity compared to ang II alone. As demonstrated in Fig. 4 the

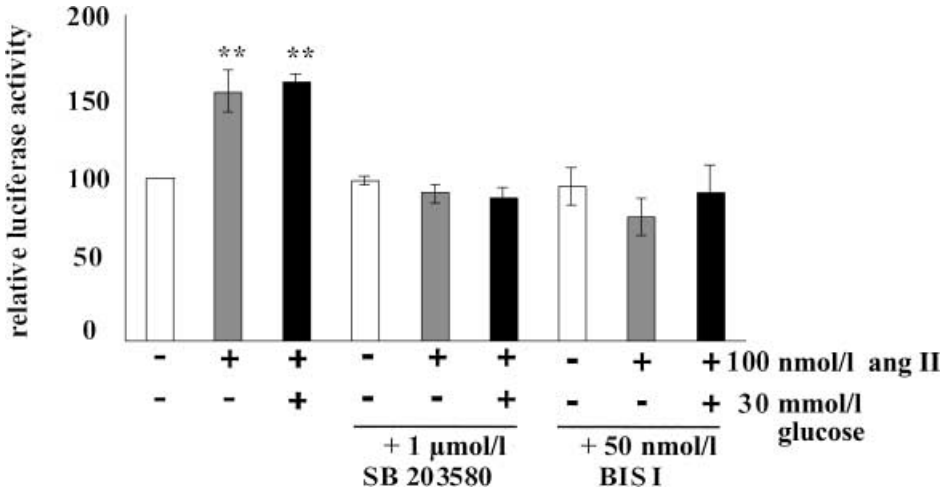

Fig. 4. Effect of high glucose concentrations and effect of inhibitors of PKC and p38 MAPK on ang II-induced TGF-β1 promoter activity. Cells were transfected with pGL3/453 and cultured for 24 h in 6 mmol/l glucose with addition of ang II or/and 24 mmol/l glucose (to receive a concentration of 30 mmol/l glucose) in the absence or presence of 50 nmol/l bisindolylmaleimide I (BIS I) or 1 µmol/l SB 203580 as indicated. Promoter activity in 6 mmol/l glucose without inhibitors was set as 100%. Data are means ± SEM of three different experiments. ***p*<0.01 vs control

Table 1. Effect of TGF-β1 promoter mutations on ang II- and high glucose-induced TGF-β1 promoter activity

	Control	ang II[a)]	high glucose[b)]
wt	100	160±16*	166±15*
Amut	34±10	23±6	28±5
Bmut	48±8	44±10	45±2
ABmut	23±3	19±2	18±4
Sp1mut	55±5	81±7*	81±14*
wt+50 nmol/l BIS I	100±2	91±6	100±18
wt+1 µmol/l SB 203580	96±12	77±11	117±17

Activation of the TGF-β1 promoter wildtype fragment –453/+11 and mutated promoter-constructs (Fig. 1) induced by 100 nmol/l ang II or 30 mmol/l glucose are shown as relative luciferase activities. BIS I and SB 203580 are PKC and p38 MAPK inhibitors, respectively

[a] Results from Figs. 2, 3, 4

[b] Results obtained with high glucose with mesangial cells from the same cell preparation

**p*<0.05 vs control

transfection experiments showed that under both conditions the promoter activity is up-regulated about 1.6-fold with no additive effect when both activators were present. Because ang II is known to activate PKC and p38 MAPK in various cells and both pathways were implicated in an increased TGF-β1 promoter activity by high glucose, we studied the effect of inhibitors of these pathways on the gene TGF-β1 activation in mesangial cells. Addition of 50 nmol/l bisindolylmaleimide I or 1 µmol/l SB 203580 prevented the promoter up-regulation completely, both with ang II alone and in the presence of both stimuli. The basal promoter activity was not affected by either inhibitor. These results indicate that ang II stimulates the TGF-β1 gene activation through PKC- and p38 MAPK-dependent pathways similar to the previously described stimulation by high glucose [34].

Ang II stimulates the binding of nuclear proteins to the AP-1 box B with no further increase by simultaneous addition of high glucose concentrations. The mutation of each of the two AP-1 binding sites in the TGF-β1 promoter fragment –453/+11 abolished the up-regulation of the promoter activity completely (Fig. 3). In previous studies we have demonstrated that AP-1 box A is only a weak binding site for the AP-1 complex, while an AP-1 complex binds specifically and glucose-dependent to AP-1 box B [34]. Furthermore, since expression of AP-1 in mesangial cells activates the transcription driven by a promoter fragment containing AP-1 box B, while the presence of AP-1 box A alone is not sufficient to mediate transcriptional activation (C. Weigert, unpublished data) we studied only the binding of nuclear proteins to this functional AP-1 binding site B by EMSA. As shown in Fig. 5A stimulation of the mesangial cells with 100 nmol/l ang II or 30 mmol/l glucose activates the binding to AP-1 box B to a similar extend (lane 2 and 3). The effect of ang II or high glucose was specific for AP-1, since the binding of nuclear proteins to the Sp1 binding site of the TGF-β1 promoter was not affected (Fig. 5B). Co-incubation of the mesangial cells with 100 nmol/l ang II and 30 mM glucose did not further activate the binding to AP-1 box B compared with ang II alone (Fig. 5C, lane 2 and 3). Quantification of the specific shifted bands showed a twofold stimulation of the DNA binding activity under both conditions (Fig. 5D). In the presence of losartan the ang II-stimulated increase in AP-1 binding activity was reduced to control levels (Fig. 5C, lane 4). The specific binding was identified by the addition of a 10-fold molar excess of cold AP-1 box B oligonucleotide (Fig. 5C,

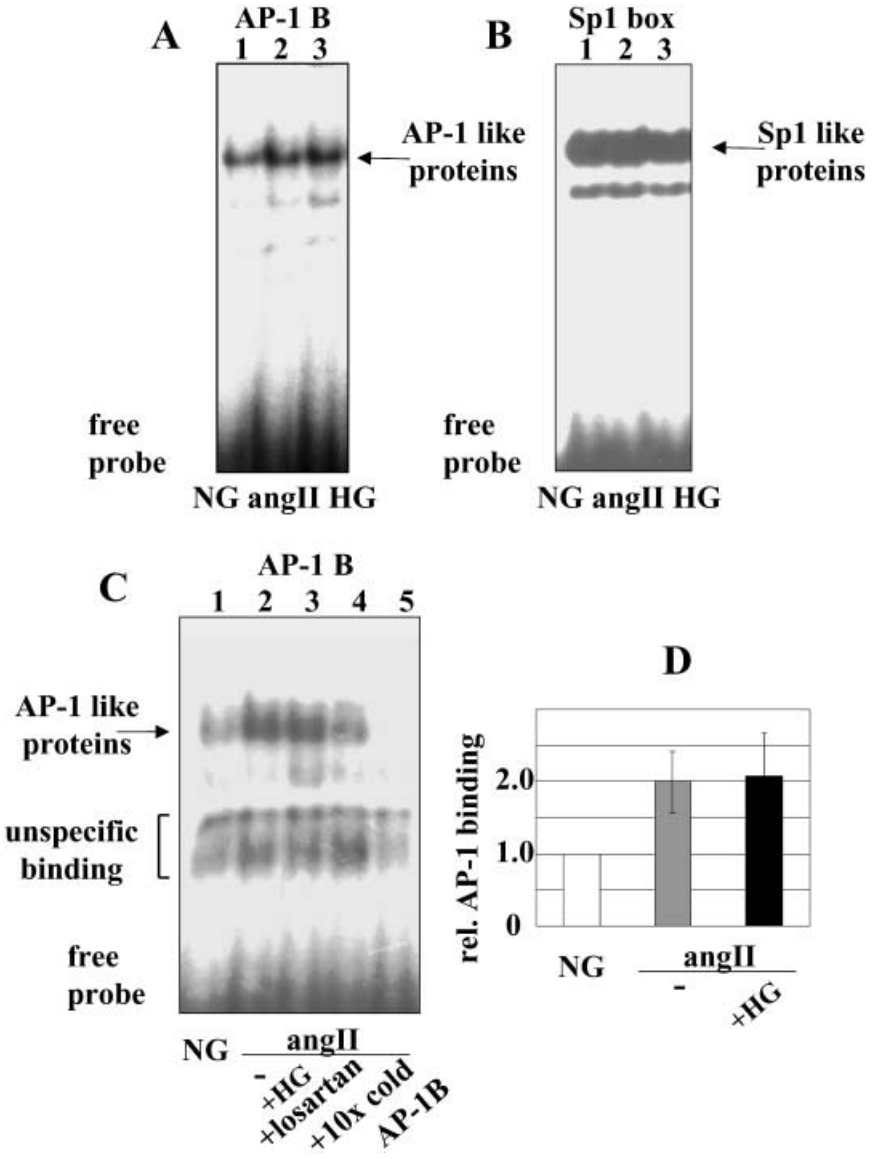

Fig. 5A–D. Effect of ang II and high glucose stimulation on the binding of nuclear proteins to AP-1 box B. Mobility shift experiments were performed with nuclear extracts from mesangial cells. Cells were cultured for 24 h and subsequently activated with 100 nmol/l ang II, 30 mmol/l glucose or both, for 24 h. 10 µg of nuclear proteins were incubated with 50 000 cpm of the ^{32}P-labelled oligonucleotides, which include the AP-1 box –371/–364 or the Sp1 site –220/–211 of the TGF-β1 promoter. **A** Binding of nuclear proteins from normal glucose- (NG), ang II-, and high glucose- (HG) treated cells to the AP-1 site B. **B** Binding of nuclear proteins from normal glucose, ang II-, and high glucose-treated cells to the Sp1 binding site –220/–211 of the TGF-β1 promoter. **C** Binding of nuclear proteins from mesangial cells incubated with normal glucose, ang II alone, or ang II and high glucose, ang II and losartan, ang II and a 10-fold molar excess of cold AP-1 B oligonucleotide to AP-1 box B, lanes 1–5 respectively. Specific binding is indicated by an *arrow*. **D** Scanning densitometry of three independent mobility shift experiments with the AP-1 box B and nuclear extracts of ang II-, and ang II- and high glucose-stimulated cells. The intensity of the specific shifted bands of unstimulated cells was set as 1.0. Data are means ± SEM

lane 5). The upper band indicated by the arrow disappeared completely while the lower bands were only marginally affected.

Ang II activates the p38 MAPK pathway in mesangial cells with no further stimulation by high glucose. Because both, ang II and high glucose, are activators of the p38 MAPK pathway, we studied a possible additive effect by quantification of the phosphorylated form of ATF-2 an endogenous substrate of the p38 MAPK pathway. In nuclear extracts of mesangial cells phosphorylated ATF-2 protein was detected by immunoblotting and quantified (Fig. 6). Ang II increased the level of phosphorylation approximately 2.5-fold, with no further increase after co-stimulation with high glucose. Again, no additive effect of ang II and high glucose could be detected.

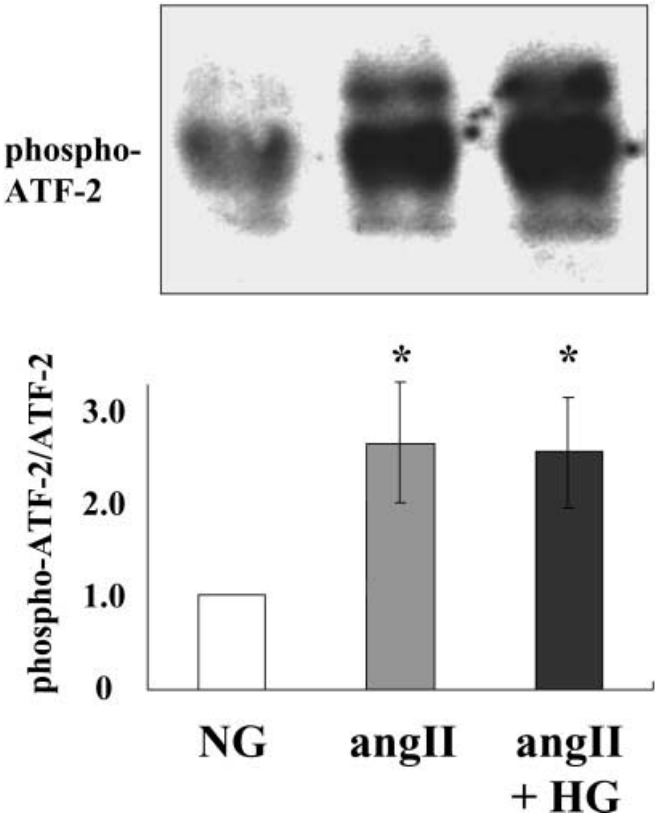

Fig. 6. Ang II and high glucose stimulation of the p38 MAPK pathway. p38 MAPK activity was assessed by phosphorylation of ATF-2. Western blot analyses were performed as described in Methods using 20 μg of nuclear proteins from mesangial cells incubated with ang II and 30 mmol/l glucose for 24 h. Phosphorylation of ATF-2 (as shown in the upper part of the figure) and ATF-2 protein were detected with specific antibodies. *Bar graphs* show densitometric quantification of the ratio of phospho-ATF-2/ATF-2 protein. The mean value of control (6 mmol/l glucose) is defined as 1. Each value is expressed as means ± SEM of three independent experiments. *$p<0.05$ vs 6 mmol/l glucose

Discussion

Previous studies have indicated that ang II exerts several non-haemodynamic effects on renal cells similar to those observed with increased glucose concentrations, including cellular responses that could lead to sclerosis such as the enhanced expression of TGF-β1 and the increased synthesis of extracellular matrix proteins. In this study we investigated the molecular mechanism by which ang II induces TGF-β1 gene expression in mesangial cells. Our main result was that ang II enhances the TGF-β1 gene activation by PKC- and p38 MAPK-dependent pathways and activation of AP-1 which in turn induces TGF-β1 promoter activation through AP-1 box A and B. Firstly, using promoter reporter constructs, we found that ang II stimulated the activation of the TGF-β1 promoter to a similar extent as previously found for high glucose-induced stimulation. Secondly, the stimulatory effect is mediated by the two AP-1 binding sites in the promoter, which also confer to the glucose-response of this promoter fragment, as shown by mutation of the AP-1 binding sites. Thirdly, the activation of the TGF-β1 promoter by ang II is mediated through PKC- and p38 MAPK-dependent pathways as shown by the addition of inhibitors of these pathways in the transfection experiments. Activation of PKC and p38 MAPK is also responsible for the high glucose-mediated TGF-β1 promoter up-regulation [34]. The participation of these pathways in both conditions is clearly shown by the complete blockade of the promoter activation by PKC or p38 MAPK-inhibitors in the presence of ang II and high glucose. Fourth, ang II stimulates the DNA binding activity of mesangial nuclear extracts to the AP-1 box B of the promoter to a similar extend as high glucose concentrations do.

Our data demonstrate for the first time the detailed molecular mechanism of the TGF-β1 promoter activation by ang II in mesangial cells by identifying the regulatory DNA elements. The involvement of AP-1 in ang II-stimulated TGF-β1 gene transcription has been suggested in previous studies with smooth muscle cells [30] and proximal tubular cells [38]. Recent results showing that AP-1 was activated in the glomerulum of ang II-infused rats [39] indicate that the presently shown ang II-induced AP-1 activation in mesangial cells occurs also in vivo.

The second main observation of our study is that high glucose concentrations had no additive effect on the ang II-stimulated promoter activity. This was accompanied by no further increases in the binding of nuclear proteins to the AP-1 site in the TGF-β1 promoter and in the activation of the p38 MAPK pathway as assessed by endogenous ATF-2 phosphorylation when both high glucose and ang II were present. Together these data indicate that ang II and high glucose use the same pathway to stimulate TGF-β1 gene expression in mesangial cells.

In a recent study with vascular smooth muscle cells, an additive response has been described after stimulation with high glucose and ang II on p38 MAPK activity and AP-1 DNA binding [26]. However, the experimental design was different. While the concentrations used were similar to ours (25 mmol/l glucose and 100 nmol/l ang II), the incubation times were different ie. the cells were preincubated with high glucose for 1 or 10 days and then stimulated for 5 min or 2 h with ang II. Because we found from studying the time dependence of the ang II-or high glucose-effect for 48 h in the mesangial cells the strongest induction of AP-1 DNA binding activity after 24 h, we used this condition to investigate a possible additive effect of both substances. Furthermore, to determine the AP-1 DNA binding activity a synthetic AP-1 consensus oligonucleotide was used differing from the sequences of the AP-1 sites in the TGF-β1 promoter.

Our study which was focused on the ang II effect on TGF-β1 promoter activation (fragment –453/+11) in mesangial cells does not exclude that high glucose and ang II might have additive effects on other cellular functions. For example long-term incubation of mesangial cells with 30 mmol/l glucose (for 5 days) resulted in an ang II-dependent inhibition of mesangial matrix degradation suggested to be mediated by an increased ang II generation of the mesangial cells [40]. Furthermore, in vivo studies provide evidence for the activation of the renal renin-angiotensin system by hyperglycaemia, which might not occur in cultured mesangial cells [41].

Together with previous results our data indicate that two major stimuli ang II and glucose, both shown to be higher in diabetic patients, activate signalling pathways which lead to TGF-β1-mediated increased matrix protein synthesis. The causal involvement of TGF-β in the development of diabetic nephropathy has been convincingly demonstrated because chronic treatment with neutralising anti-TGF-β antibody in diabetic rats prevented early changes of renal hypertrophy and excess matrix gene expression [13]. From these animal experiments it is not readily obvious if hyperglycaemia and/or higher ang II are responsible for the increased renal TGF-β activity. Several studies in diabetic patients and in experimental animals indicate that inhibition of ang II action, by ACE inhibitors or by ang II receptor antagonists, provide renoprotective effects independent of the reduction of the blood pressure [8, 9, 10].

In summary, we have identified ang II responsive elements on the TGF-β1 promoter and we have shown that ang II and high glucose use the same pathway to enhance TGF-β1 gene activation in mesangial cells. This might be important for a possible therapeutical intervention because the blockade of the common high glucose/ang II-activated signalling pathway could prevent not only the adverse effects of ang II (without affecting the blood pressure), but also the action of sustained hyperglycaemia. Furthermore, our data suggest the molecular pathogenesis of ang II-mediated induction of hypertensive nephro-angiosclerosis.

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