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Angiotensin II stimulation of the stress-activated protein kinases in renal mesangial cells is mediated by the angiotensin AT_1 receptor subtype

Andrea Huwiler [a], Gerda van Rossum [a], Markus Wartmann [b], Josef Pfeilschifter [c,*]

[a] *Center for Biomembranes and Lipid Enzymology, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands*
[b] *Research Department, Pharmaceutical Divisions, Novartis Ltd., 4002 Basel, Switzerland*
[c] *Zentrum der Pharmakologie, Klinikum der Johann Wolfgang Goethe-Universität, Theodor-Stern-Kai 7, 60590 Frankfurt am Main, Germany*

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Abstract

Treatment of renal mesangial cells with the vasoconstrictor angiotensin II stimulates a concentration-dependent increase in stress-activated protein kinase (SAPK) activity as measured by phosphorylation of the substrate c-Jun. Time course studies reveal a transient SAPK activation by angiotensin II which is maximal after 5–10 min of stimulation and rapidly declines thereafter to basal levels within 30 min. Using the highly selective angiotensin II AT_1 receptor antagonist valsartan, a concentration-dependent inhibition of angiotensin II-induced SAPK activity is observed, clearly implying the AT_1-receptor in this angiotensin II-mediated response. To further elucidate the mechanism involved in angiotensin II-induced SAPK activation, cells were treated with different inhibitors. Genistein, a tyrosine kinase inhibitor, greatly blocks (by 90%) the angiotensin II response, whereas pertussis toxin only partially inhibits angiotensin II-activated SAPK activity (by 76%). A highly potent protein kinase C inhibitor {3-[1-[3-(amidinothio)propyl-1H-indoyl-3-yl]-3-(1-methyl-1H-indoyl-3-yl) maleimide methane sulfonate}, Ro 31-8220, as well as protein kinase C depletion from the cells by prolonged phorbol ester pretreatment, fail to inhibit the angiotensin II-induced SAPK activation. In summary these results suggest that angiotensin II AT_1-receptor is able to activate the SAPK cascade in mesangial cells by a pathway independent of protein kinase C, but requiring both pertussis-toxin-sensitive and -insensitive G-proteins and tyrosine kinase activation. © 1998 Elsevier Science B.V.

Keywords: Angiotensin II; Angiotensin AT_1 receptor; Stress-activated protein kinase; Mesangial cell

1. Introduction

Mesangial cells are a major determinant in the regulation of the glomerular filtration rate. Morphologically, mesangial cells resemble vascular smooth muscle cells and are able to contract upon stimulation by vasoactive hormones like angiotensin II or arginine vasopressin (Menè et al., 1989; Pfeilschifter, 1989).

Angiotensin II is involved in a variety of physiological functions and regulates vascular tone, glomerular haemodynamics, tubular transport and also chemoattraction of cells, mesangial cell processing of macromolecules, immunomodulation, angiogenesis and growth regulation (Bottari et al., 1993; De Gasparo and Levens, 1994).

Mechanistically, angiotensin II binds to specific surface receptors on mesangial cells (Pfeilschifter, 1990a) and activates via a G-protein, a phospholipase C which hydrolyses phosphatidylinositol 4,5-bisphosphate to inositol 1,4,5-trisphosphate and 1,2-diacylglycerol. Inositol 1,4,5-trisphosphate mobilizes Ca^{2+} from the endoplasmatic reticulum, while diacylglycerol activates protein kinase C (Menè et al., 1989; Pfeilschifter, 1989). By the development of specific peptidic and non-peptidic angiotensin II receptor antagonists two subtypes of angiotensin II receptors were identified (Chiu et al., 1989; Whitebread et al., 1989; Smith and Timmermans, 1994), the AT_1 type and the AT_2 type. The former one has recently been shown to mediate angiotensin II-stimulated phosphoinositide turnover (Pfeilschifter, 1990a) and angiotensin II-stimulated phospholipase D activation in mesangial cells (Pfeilschifter et al., 1992). In contrast, the signalling pathways triggered by the angiotensin II AT_2 receptor are still poorly defined, but do not include the aforementioned classical messenger pathway. Reportedly, angiotensin II AT_2 receptors do not interact with guanine nucleotide-binding proteins, thus excluding all the signalling cascades

* Corresponding author. Tel.: +49-69-63016951; fax: +49-69-63017942; e-mail: pfeilschifter@em.uni-frankfurt.de

involving G-proteins. (Bottari et al., 1991; Pucell et al., 1991).

In mammals, three structural families of mitogen-activated protein (MAP) kinase have been identified that have distinct substrate specificities (Cano and Mahadevan, 1995; Woodgett et al., 1996). The members of one family, the extracellular signal-regulated kinase (ERK)-1 and ERK-2, also named p44 and p42 mitogen-activated protein kinases (mapk), are activated primarily by mitogenic agonists. The c-Jun N-terminal kinase (JNK) family, also known as stress-activated protein kinases (SAPK) and the $p38^{mapk}$/reactivating kinase (RK), which is the closest structural homolog of the yeast HOG1, are activated either in common or parallel pathways by cellular stresses such as interleukin-1 and tumour necrosis factor-α, heat shock, UV-light, osmotic shock and metabolic poisons and to a lesser extent by growth factors.

In this study we show that mesangial cells respond to angiotensin II stimulation with increased SAPK activity and that this angiotensin II effect is mediated by the AT_1-receptor subtype in a protein kinase C-independent, but tyrosine kinase-dependent manner.

2. Materials and methods

2.1. Chemicals

Angiotensin II, 12-*O*-tetradecanoyl-phorbol 13-acetate (TPA) and genistein were purchased from Calbiochem, Lucerne, Switzerland; [^{32}P]ATP and Hyperfilm MP were from Amersham International, Amersham, Bucks., UK; glutathione-sepharose was from Pharmacia Fine Chemicals, Uppsala, Sweden; CGP 42 112A (angiotensin II-(4–8)-peptide), valsartan and glutathione *S*-transferase-coupled c-Jun were synthesized and prepared by Ciba-Geigy, Basel, Switzerland; pertussis toxin was from Sigma; *N*-acetyl-cysteine was from Fluka and all cell culture nutrients were from Boehringer, Mannheim, Germany.

{3-[1-[3-(Amidinothio)propyl-1H-indoyl-3-yl]-3-(1-methyl-1H-indoyl-3-yl) maleimide methane sulfonate}, Ro 31-8220, was a generous gift of F. Hoffmann-La Roche, Basel, Switzerland.

2.2. Cell culture

Rat renal mesangial cells were cultivated as described previously (Pfeilschifter, 1990b). In a second step, single cells were cloned by limited dilution on 96-microwell plates. Clones with apparent mesangial-cell morphology were used for further processing. The cells were grown in RPMI 1640 supplemented with 10% (v/v) fetal-bovine serum, penicillin (100 units/ml), streptomycin (100 μg/ml) and bovine insulin (0.66 unit/ml). Mesangial cells exhibited the typical stellate morphology. Moreover, there was positive staining for the intermediate filaments desmin and vimentin, which is considered to be specific for myogenic cells, positive staining for Thy 1.1 antigen and negative staining for factor-VIII-related antigen and cytokeratin, which excludes endothelial and epithelial contaminations.

Generation of inositol trisphosphate upon activation of angiotensin II AT_1-type receptor (Pfeilschifter, 1990a) was used as a functional criterion for characterizing the cloned cell line. For the experiments, passages 5–15 of mesangial cells were used.

2.3. Solid phase c-Jun kinase activity assay

The solid-phase c-Jun kinase assay was performed as described previously (Hibi et al., 1993) with a glutathione *S*-transferase (GST)-c-Jun (5–89) fusion protein coupled to glutathione-sepharose beads as substrate. In brief, 10 μg of GST-c-Jun was coupled to 10 μl of glutathione-sepharose in 0.5 ml of TLB buffer (20 mM Tris–HCl, pH 7.4, 137 mM NaCl, 2 mM EDTA, 1% Triton X100, 1 mM Na_3VO_4, 2 mM sodium pyrophosphate, 1 mM phenylmethanesulfonyl fluoride, 10 μg/ml leupeptin, 25 mM β-glycerophosphate) for 30 min at 4°C. The beads were then centrifuged for 20 s at $14\,000 \times g$, washed twice with TLB buffer and incubated for 2 h at 4°C with cell extracts containing 200 μg of protein. Thereafter, the complexes were washed twice with TLB buffer and once with 20 mM HEPES, pH 7.4, 20 mM $MgCl_2$ before the kinase reaction was started by addition of 30 μl of kinase buffer (20 mM HEPES, pH 7.4, 20 mM $MgCl_2$, 2 mM dithiothreitol, 20 mM β-glycerophosphate, 10 μM ATP and 2 μCi [^{32}P]ATP to the complexes and incubated for 20 min at 30°C. To stop the reaction, 30 μl of 2× Laemmli sample buffer was added and the samples were heated for 5 min at 95°C. Proteins were separated by sodium dodecylsulfate (SDS) polyacrylamide gel electrophoresis (PAGE) (13% acrylamide gel) and phosphorylated GST-c-Jun was detected by autoradiography and quantitated by a phosphorimager (Molecular Dynamics).

2.4. Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA). For multiple comparisons with the same control group, the limit of significance was divided by the number of comparisons according to Bonferroni. IC_{50} values were derived by using a double-reciprocal regression plot as described by Kenakin (1984).

3. Results

Previously it was reported that ERK and SAPK families phosphorylate Ser-63 and Ser-73 of the N-terminal transactivation domain of c-Jun in vitro (Alvaresz et al., 1991). In

contrast to ERKs which do not bind c-Jun with high affinity (Hibi et al., 1993; Gupta and Davis, 1994), the SAPKs bind to c-Jun and may be responsible for phosphorylation of c-Jun in vivo (Kallukini et al., 1994). We used a solid-phase kinase assay with c-Jun (5–89) coupled to glutathione *S*-transferase as a substrate that binds all SAPK isoforms and after precipitation with glutathione-sepharose the N-terminal domain of c-Jun is phosphorylated by activated SAPKs (Hibi et al., 1993). Previously we demonstrated by Western blot analysis, that mesangial cell lysates express p46 SAPKα and p54 SAPKγ, whereas the β-isoform could not be detected (Huwiler et al., 1997). Hence, both isoforms, α and γ, may contribute to the SAPK activity measured in this study.

The data in Fig. 1 show that angiotensin II rapidly augmented SAPK activity within 2–5 min. Maximal activation was reached between 5 and 10 min. Thereafter the activity rapidly declined and at 30 min it no longer differed from control values. Up to 60 min, the latest time point examined, no further increase in SAPK activity could be detected. Anisomycin (50 nM) is included in Fig. 1 as a positive control to compare the extent of SAPK activation by angiotensin II.

Fig. 2 demonstrates the concentration-dependency of SAPK activation induced by angiotensin II after 10 min of stimulation. Potent activation of SAPK was detected already at 10 nM of angiotensin II and a maximal response was obtained at 100 nM of angiotensin II. At higher concentrations (1 μM) SAPK activation by angiotensin II declined again (Fig. 2).

In order to assess which angiotensin II receptor subtype is involved in SAPK activation, the effect of valsartan, a specific non-peptidic angiotensin II AT_1-receptor antagonist was investigated (Criscione et al., 1993). Valsartan potently blocked angiotensin II-stimulated c-Jun phosphorylation (Fig. 3) with an IC_{50} of 1.8 nM, whereas the AT_2 receptor-specific ligand CGP 42112A (100 nM) had no inhibitory effects (data not shown), thus, clearly indicating that the angiotensin II AT_1 receptor triggers SAPK activation in mesangial cells. This conclusion is further supported by the potent stimulating action of angiotensin III on SAPK activity which is comparable in its potency to angiotensin II as shown in Fig. 4 (Chiu et al., 1990).

To further elucidate the mechanism underlying the angiotensin II-mediated SAPK activation, genistein, an isoflavone compound from fermentation broth of *Pseudomonas spp.* and a widely used tyrosine kinase inhibitor (Akiyama et al., 1987), was tested for its ability to inhibit SAPK activity. As shown in Fig. 5, genistein completely blocked angiotensin II-induced SAPK activity at a concentration of 50 μM, clearly suggesting an important role of tyrosine kinases in SAPK activation.

To assess the role of protein kinase C in angiotensin II-stimulated SAPK activation, a highly selective protein kinase C inhibitor as well as protein kinase C depletion experiments were used. The selective protein kinase C

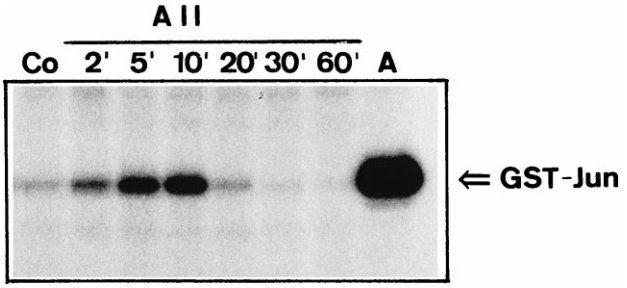

Fig. 1. Time course of angiotensin II-stimulated SAPK activity in mesangial cells. Quiescent mesangial cells were treated with vehicle (Co), angiotensin II (100 nM, AII) or anisomycin (50 nM, A) for the indicated time periods (in min). Thereafter, cells were harvested and SAPK activity was measured as described in Section 2. Similar results were obtained in two independent experiments.

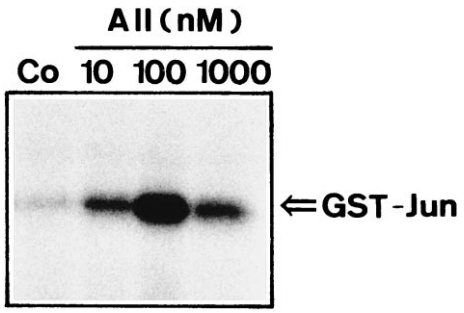

Fig. 2. Concentration-dependence of angiotensin II-stimulated SAPK activity in mesangial cells. Quiescent mesangial cells were treated with either vehicle (Co) or the indicated concentration of angiotensin II (AII) for 10 min. Cells were harvested and 200 μg of cell protein was taken for SAPK activity measurements as described in Section 2. Similar results are obtained in three independent experiments.

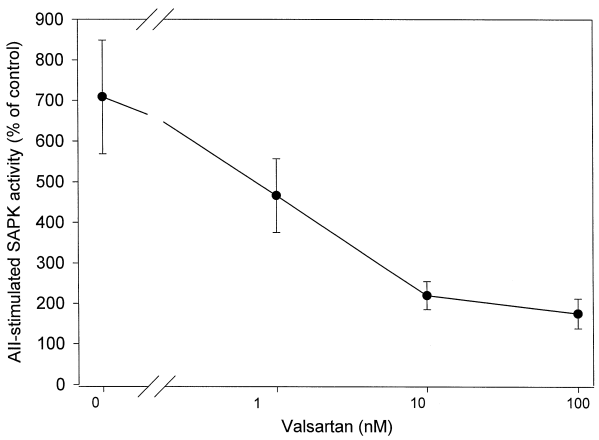

Fig. 3. Inhibition of angiotensin II-stimulated SAPK activity by valsartan. Mesangial cells were treated with vehicle (control) or angiotensin II (100 nM) in the absence or presence of the indicated concentrations of valsartan for 10 min. Cells were then harvested and SAPK activity was measured as described in Section 2. Results are means ± S.D of three independent experiments.

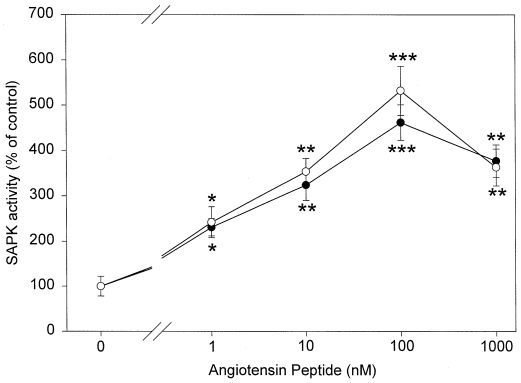

Fig. 4. Concentration-dependence of angiotensin II- and angiotensin III-stimulated SAPK activity in mesangial cells. Quiescent mesangial cells were treated with either vehicle or the indicated concentration of angiotensin II (●) or angiotensin III (○) for 10 min. Cells were harvested and 200 μg of cell protein was taken for SAPK activity measurements as described in Section 2. Similar results are obtained in three independent experiments. Significant differences from control: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ANOVA.

inhibitor Ro 31-8220, which inhibits all classical and novel protein kinase C isoforms with an average IC_{50} of 5 nM (Alessi, 1997) failed to inhibit the angiotensin II response, even at concentrations up to 1 μM. Moreover, protein kinase C downregulation by a prolonged 24 h exposure of mesangial cells to phorbol ester TPA (Huwiler et al., 1991, 1992) had no inhibitory influence on angiotensin II-stimulated SAPK activity (Fig. 5). These results indicate that SAPK activation induced by angiotensin II occurs independently of phorbol ester-sensitive protein kinase C isoforms.

Pretreatment for 20 h with pertussis toxin (100 ng/ml) caused only a partial (76%) inhibition of the angiotensin II-stimulated SAPK activation implicating that not only pertussis toxin-sensitive G-proteins (Ui, 1984), but additional pertussis-toxin-insensitive G-proteins mediate SAPK activation in angiotensin II-activated mesangial cells.

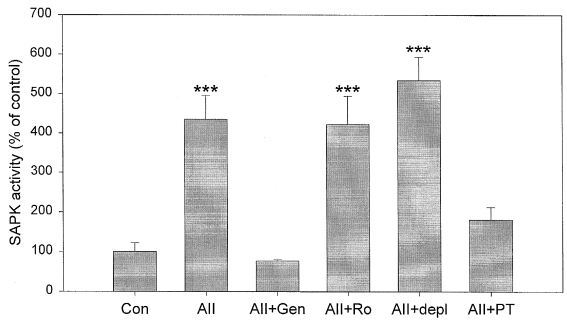

Fig. 5. Effect of various compounds on angiotensin II-stimulated SAPK activity in mesangial cells. Quiescent mesangial cells were pretreated for 24 h with TPA (500 nM, depl.) or for 30 min with Ro 31-8220 (1 μM, Ro), genistein (10 μM, Gen), or for 20 h with pertussis toxin (100 ng/ml, PT) and then stimulated with either vehicle (Con) or angiotensin II (100 nM, AII) for 10 min as indicated. Thereafter, cells were harvested and SAPK activity was measured as described in Section 2. Results are means ± S.D. of three independent experiments. Significant differences from control: $^{***}P < 0.001$; ANOVA.

4. Discussion

Our results demonstrate that angiotensin II evokes a very rapid and transient activation of the SAPK cascade in mesangial cells. The potent inhibitory action of valsartan on A II-induced SAPK activity clearly demonstrates that this effect is mediated by the angiotensin II AT_1-receptor. The angiotensin II AT_1 receptor has been cloned and Northern blot analysis has revealed that the mRNA for this receptor subtype is expressed in bovine adrenal medulla and cortex, kidney and vascular smooth muscle cells (Murphy et al., 1991; Sasaki et al., 1991). This receptor subtype has been shown to mediate most known effects of angiotensin II by coupling via G-proteins to phosphoinositide hydrolysis, stimulation of calcium mobilization, activation of a phosphatidylcholine-degrading phospholipase D and inhibition of adenylate cyclase (Chiu et al., 1990; Garcia-Sainz and Macias-Silva, 1990; Pfeilschifter and Bauer, 1986; Pfeilschifter, 1990a; Bauer et al., 1991; Pfeilschifter et al., 1992). Furthermore, the AT_1 receptor is involved in angiotensin II-mediated p42 and p44 MAPK activation (Huwiler et al., 1995 and unpublished results). Our data now also include the SAPK cascade as an additional target for angiotensin II signalling via AT_1 receptor activation thus confirming a report by Zohn et al. (1995) on angiotensin II activation of JNK activity which however has not defined the responsible receptor subtype.

In contrast to these angiotensin II AT_1 receptor-mediated responses, the signalling pathways initiated by the angiotensin II AT_2 receptor are still unclear. Recent reports indicate that there is no interaction between angiotensin II AT_2 receptor and G-proteins thus excluding all signalling cascades involving G-proteins (Bottari et al., 1991; Pucell et al., 1991).

Since angiotensin II couples via the AT_1 receptor to heterotrimeric G-proteins and triggers phosphoinositide hydrolysis with subsequent activation of protein kinase C, we addressed the role of protein kinase C in triggering SAPK activation. Previously, we reported that mesangial cells express four protein kinase C isoenzymes, -α, -δ, -ε and -ζ. No protein kinase C-β1, -β2, -γ, -η, -θ and -μ are expressed (Huwiler et al., 1991; Huwiler et al., 1992; Pfeilschifter and Huwiler, 1997). Upon 24 h phorbol ester treatment, all phorbol ester-sensitive isoforms, protein kinase C-α, -δ and -ε, can be downregulated (Huwiler et al., 1991; Huwiler et al., 1992). Here we could demonstrate that such a 24 h phorbol ester treatment did not reduce the SAPK activity triggered by angiotensin II. In line with these findings the specific protein kinase C inhibitor Ro 31-8220 (Davis et al., 1989) had no inhibitory effect on angiotensin II-induced SAPK activation. In summary, these data suggest that at least the phorbol ester-sensitive protein kinase C isoforms, α, δ and ε, are not involved in angiotensin II-mediated SAPK activation. However, an involvement of the phorbol ester-insensitive protein kinase C-ζ can not be ruled out from these experiments. It is

noteworthy that mesangial cells depleted of phorbol ester-sensitive protein kinase C isoenzymes respond to angiotensin II stimulation with an increased SAPK activity (Fig. 5) when compared to cells not pretreated with phorbol ester, an observation also noted in rat liver epithelial cells (Zohn et al., 1995). This may be due to removal of a negative regulatory effect exerted by protein kinase C at the level of the angiotensin II AT_1 receptor or the coupling of G-proteins (Pfeilschifter, 1994).

Interestingly, angiotensin II is a potent activator of both SAPK and classical ERK cascades (Huwiler et al., 1995). Originally it was thought that SAPK cascades are preferentially activated by cellular stresses such as protein synthesis inhibitors, UV irradiation, inflammatory cytokines or genotoxic agents (Cano and Mahadevan, 1995; Woodgett et al., 1996). However, recent evidence indicates that also G-protein coupled receptors, that classically trigger the ERK pathway, are also delivering signals to the SAPK signalling module. Coso et al. (1996) have reported that activation of m1 and m2 muscarinic receptors overexpressed in COS-7 cells (CV1 origin simian virus 40 transformed monkey cells) increases JNK activity. In addition, endothelin 1 has been demonstrated to stimulate JNK activity in rat ventricular myocytes (Bogoyevitch et al., 1995) and in Rat-1 cells (Cadwallader et al., 1997) and α_1-adrenergic receptor stimulation causes JNK activation in rabbit aortic smooth muscle cells (Nishio et al., 1996).

Whereas the angiotensin II-induced SAPK activation is independent of protein kinase C, the ERK cascade stimulated by angiotensin II depends on protein kinase C activation, in particular on a Ca^{2+}-independent novel protein kinase C isoenzyme (Huwiler and Pfeilschifter, unpublished results). Such a differential regulation of the ERK and SAPK pathways triggered by the same agonist underlines the importance of understanding the cross-communication between the SAPK and ERK pathways. An integration of both signals will determine the cell's fate upon angiotensin II treatment. Recent observations by Gotoh et al. (1990) and Traverse et al. (1992) in the rat pheochromocytoma cell line PC 12 emphasize the crucial role of the magnitude and duration of ERK activity in determining functional cell responses. Moreover, we have reported that mesangial cells undergo hypertrophy but do not proliferate upon chronic angiotensin II exposure due to the only transient activation of the ERK pathway (Huwiler et al., 1995).

We are presently examining the interplay between ERK and SAPK cascades in mesangial cells and its role in determining functional cell responses relevant to physiology and pathophysiology of the renal glomerulus.

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