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Angiotensin converting enzyme inhibitors block mitogenic signalling pathways in rat cardiac fibroblasts

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Abstract We studied the effects of angiotensin converting enzyme (ACE) inhibitors on angiotensin II (Ang II) induced growth related signalling pathways in neonatal rat cardiac fibroblasts. In BrdU proliferation assays, Ang II (10^{-9}–10^{-7} M) stimulated cardiac fibroblast growth in a dose-dependent fashion (maximum at 10^{-7} M, 5.22 ± 0.01-fold, $n = 9$). 2-2-(1-(ethoxycarbonyl)-3-phenylpropyl)-[amino-oxopropyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3 carboxylic acid (moexiprilat) led to a dose-dependent inhibition of the Ang II induced cardiac fibroblast growth. A less pronounced effect on cellular proliferation was seen with the ACE inhibitor enalaprilat. To elucidate the mechanisms involved in this direct antiproliferative effect of ACE inhibitors in cardiac fibroblasts, we studied the activation of mitogen-activated protein kinases [MAPKs: extracellular signal-regulated kinases 1 and 2 (ERK1/2) and p38-MAPK] and JAK/STAT (janus kinases/signal transducer and activator of transcription) signal transduction pathways. Ang II (10^{-7} M) caused an increase in MAPKs activity with an increased phosphorylation of ERK1/2 (1.7-fold) and p38-MAPK (3.6-fold). This effect was completely inhibited by moexiprilat (10^{-7} M) and enalaprilat (10^{-7} M). Stimulation with Ang II (10^{-7} M) also led to an increased phosphorylation of STAT3, which is one of the key effector proteins in the JAK/STAT signalling pathway. This effect was also completely inhibited by moexiprilat (10^{-7} M) and enalaprilat (10^{-7} M). These data show that the ACE inhibitors moexiprilat and enalaprilat inhibit Ang II induced proliferation of cardiac fibroblasts according to their relative potency of ACE inhibition in vitro. This novel effect of ACE inhibitors is accompanied by blocking the Ang II induced activation of several intracellular signal transduction pathways (ERK1/2, p38-MAPK and STAT3).

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Introduction

Angiotensin II (Ang II) has been attributed to play a major role in the pathogenesis of left ventricular hypertrophy and cardiac fibrosis in patients with hypertensive heart disease. On the one hand, systemic effects of Ang II are mediated by the increased blood pressure, which leads to left ventricular hypertrophy via the increased mechanical loading (Dahlöf et al. 1994; Hefti et al. 1997). On the other hand, increased levels of Ang II in patients with arterial hypertension directly stimulate cardiac fibroblast growth, leading to cardiac fibrosis (Booz and Baker 1995; Brown and Vaughan 1998; Weber 1997). Angiotensin converting enzyme (ACE) inhibitors are well established in the treatment of hypertensive heart disease, as they have been shown to reverse left ventricular hypertrophy (Dahlöf et al. 1992; Gottdiener et al. 1997) and influence cardiac remodeling after myocardial infarction (Pfeffer 1995; Schieffer et al. 1994; Taylor et al. 1998). These effects of ACE inhibitor treatment are thought to be mediated by the reduction of circulating levels and tissue concentrations of Ang II. Several studies have shown that different ACE inhibitors are able to abolish the effects of externally administered angiotensin I (Ang I) in vivo and in vitro. Treatment with the ACE inhibitor 2-2-(1-(ethoxycarbonyl)-3-phenylpropyl)-[amino-oxopropyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3 carboxylic acid (moexiprilat) inhibits the rise in arterial blood pressure after intravenous application of Ang I in normotensive rats and reduces Ang I induced contractions of rabbit aorta (Friehe and Ney 1997). On the cellular level the ACE inhibitor enalaprilat blocks the Ang I induced expression of the L-type dependent calcium channel (L-VDCC) and the Na/Ca-exchanger in rat hearts (Kritzanova et al. 1997). Recent studies have shown that ACE inhibition with moexiprilat

(White et al. 1994) leads to the regression of Ang II mediated cardiac fibroblast growth by inhibition of Ang II stimulated immediate early gene expression (Grohé at al. 1997, 1998). This gives rise to the hypothesis that moexiprilat has a direct antiproliferative effect on cardiac fibroblast growth beyond the reduction of Ang II levels. However, the critical mitogenic signalling pathways involved in this process remain to be elucidated. As the mitogen activated protein kinases (MAPKs; Schorb et al. 1993, 1995; Zou et al. 1998) and the signal transducer and activator of transcription 3 (STAT3; Bhat et al. 1994, 1995) have been identified as possible mediators of Ang II induced cardiac fibroblast growth (Booz and Baker 1995), we studied the effects of ACE inhibition on Ang II induced cardiac fibroblast growth, activation of MAPKs and STAT3 phosphorylation.

Materials and methods

Cell preparation. Isolation of rat cardiac fibroblasts from neonatal rats was performed according to a modified protocol as described previously (Grohé et al. 1997; Simpson and Savion 1982). Briefly, the hearts of 1–2 day old rats (Wistar-Kyoto) were isolated and digested with 10 ml of Spinner-solution (116 mM NaCl, 5.3 mM KCl, 8 mM NaH_2PO_4, 22.6 mM $NaHCO_3$, 10 mM HEPES, 5 mM D-glucose, pH 7.4) containing 0.1% collagenase (Cytogen, Berlin, Germany) for 10 min at 37 °C in eight consecutive steps. After each digestion, the medium containing the suspended cells was removed and an equal volume of Spinner/collagenase-solution was added. The cardiac cell suspension was mixed with an equal volume of Ham's F10 (Gibco BRL, Eggenstein, Germany) supplemented with 10% horse serum (HS; Biochrom, Berlin, Germany), 10% fetal calf serum (FCS; c.c.pro, Hamburg, Germany) and 25 µg/ml gentamycin (Gibco BRL, Eggenstein, Germany) and stored at 4 °C. Cells were centrifuged at 400 *g* for 5 min and the cell pellets were resuspended in 20 ml of Ham's F10 supplemented with 10% HS and 10% FCS and plated on culture dishes. After 75 min the medium which contained the cardiomyocyte fraction of the digested tissue was removed. The dishes were gently rinsed three times to remove remaining cardiomyocytes. The adherent fraction of the plated cells consisted of cardiac fibroblasts. The culture medium for cardiac fibroblasts was changed for DMEM (Gibco BRL, Eggenstein, Germany) supplemented with 20% FCS and 25 µg/ml gentamycin. Purity of cardiac fibroblast culture was assessed by repeated differential plating and microscopic evaluation.

Proliferation assay. After 24-h incubation in serum- and phenol red-free medium (DMEM), neonatal rat cardiac fibroblasts (25,000 cells/well of a 96 microtiter plate), passages 2–3, were stimulated with Ang II (10^{-9}–10^{-6} M) or angiotensin III (AngIII; 10^{-9}–10^{-6} M) and coincubated with either vehicle alone, an ACE inhibitor, moexiprilat (the bioactive form of the pharmacologic compound moexipril; 10^{-12}–10^{-6} M; White et al. 1994), enalaprilat (the bioactive compound of enalapril; 10^{-12}–10^{-6} M), an AT_1 receptor antagonist, 2-ethoxy-1-[[2′-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]1H-benzimid-azole-7-carboxylicacid (CV11974, the bioactive form of candesartan; 10^{-7} M; Ojima et al. 1997), and a MEK1 (synonymous MAPKK) inhibitor, PD98059 (10^{-6}–10^{-4} M; Bachem, Heidelberg, Germany), which inhibits the activation of ERK1/2 via MEK1 (Dudley et al. 1995), respectively. Cellular proliferation was assessed by BrdU incorporation during the last 4 h of the 24-h incubation period using a colorimetric immunoassay according to the manufacturer's guidelines (Boehringer Mannheim, Germany). The extinctions were measured at 450 nm in an elisa plate reader (Titertek multiscan plus, EFlab, Helsinki, Finland). In control experiments cells were preincubated with moexiprilat, enalaprilat, CV11974 or PD98059 for 30 min before the addition of Ang II. All values consist of an $n = 9$.

Immunoblotting. Neonatal cardiac fibroblasts, passages 2–3, were starved for 24 h in serum-free medium. After stimulation with Ang II, moexiprilat, enalaprilat or candesartan the cells were lysed in 0.5 ml of the following buffer: 50 mM NaCl, 20 mM Tris (pH 7.4), 50 mM NaF, 50 mM EDTA, 20 mM sodium pyrophosphate ($Na_4P_2O_7$), 1 mM sodium orthovanadate (Na_3VO_4), 1% triton X-100, 1 mM PMSF, 0.6 mg/ml leupeptin and 10 µg/ml aprotinin. Protein content was measured with a standard Bradford assay. Total cell lysates (40 µg/lane) were analyzed by SDS-PAGE in a 7.5% or 12% (ERK1/2 detection) gel and transferred to a nitrocellulose membrane. Immunoblotting was performed with either a monoclonal phospho-specific pERK1/2 antibody (New England Biolabs, Schwalbach/Taunus, Germany; 1 : 500 dilution), a polyclonal STAT3 antibody (New England Biolabs, Schwalbach/Taunus, Germany; 1 : 500 dilution) or a polyclonal phospho-specific pSTAT3 antibody (New England Biolabs, Schwalbach/Taunus, Germany; 1 : 500 dilution) and anti-rabbit or anti-mouse IgG horseradish peroxidase antibody (Amersham, UK; 1 : 5000 dilution) as a secondary antibody, followed by detection with the enhanced chemiluminescence technique (ECL, Amersham, UK). The phospho-specific antibodies are directed against the phosphorylated proteins and lack crossreactivity with the native proteins. Densitometrical analysis was performed on an Epson GT 8000 scanner using the analysis software ScanPack (Biometra, Göttingen, Germany).

Immunoprecipitation. Four hundred micrograms of cellular lysates (prepared as described above) were incubated with a polyclonal antibody against p38-MAPK (Santa Cruz Biotechnology, Heidelberg, Germany; 1 : 100 dilution). Cellular extracts were incubated with the antibody for 24 h at 4 °C. Forty microliters Protein A sepharose were added to the lysate followed by incubation for 3 h at 4 °C. Samples were centrifuged in a cooled microfuge (4 °C) for 2 min at full speed. Pellets were washed twice and resuspended in 20 µl total volume. SDS-loading buffer was added and samples were boiled for 5 min at 95 °C. Sepharose beads were pelleted by centrifugation in a microfuge at 12,000 *g* for 5 min at 4 °C. The supernatants were then analyzed by SDS-PAGE. Western blots were carried out using a monoclonal anti-phospho-tyrosine antibody (PY20; Santa Cruz Biotechnology, Heidelberg, Germany; 1 : 500 dilution). An anti-mouse IgG horseradish peroxidase antibody (Amersham, UK; 1 : 5000 dilution) served as secondary antibody, followed by detection with the enhanced chemiluminescence technique (ECL; Amersham, UK).

Materials. All chemicals were obtained from Merck, Darmstadt, Germany, and Sigma Chemicals, Deisenhofen, Germany, if not otherwise specified.

Statistics. All reported values are means ± SEM. Statistical comparisons were made by Student's *t*-test with adjustment for multiple comparisons. Statistical significance was assumed if a null hypothesis could be rejected at the $P < 0.05$ level.

Results

Ang II induced neonatal rat cardiac fibroblast growth, measured by BrdU incorporation assays, in a dose-dependent fashion (10^{-9} M: 2.51 ± 0.01-fold; 10^{-8} M: 3.44 ± 0.01-fold; 10^{-7} M: 5.22 ± 0.01-fold; $n = 9$; Fig. 1 a). Coincubation with the MEK1 (MAPKK) blocker PD98059 (10^{-6}–10^{-4} M), which specifically inhibits activation of ERK1/2, led to a partial inhibition (77%, 72% and 45%, respectively) of Ang II (10^{-7} M) induced cardiac fibroblast growth (Ang II + PD 10^{-6} M: 3.34 ± 0.1-fold; Ang II + PD 10^{-5} M: 2.23 ± 0.09-fold; Ang II + PD 10^{-4} M: 1.97 ±

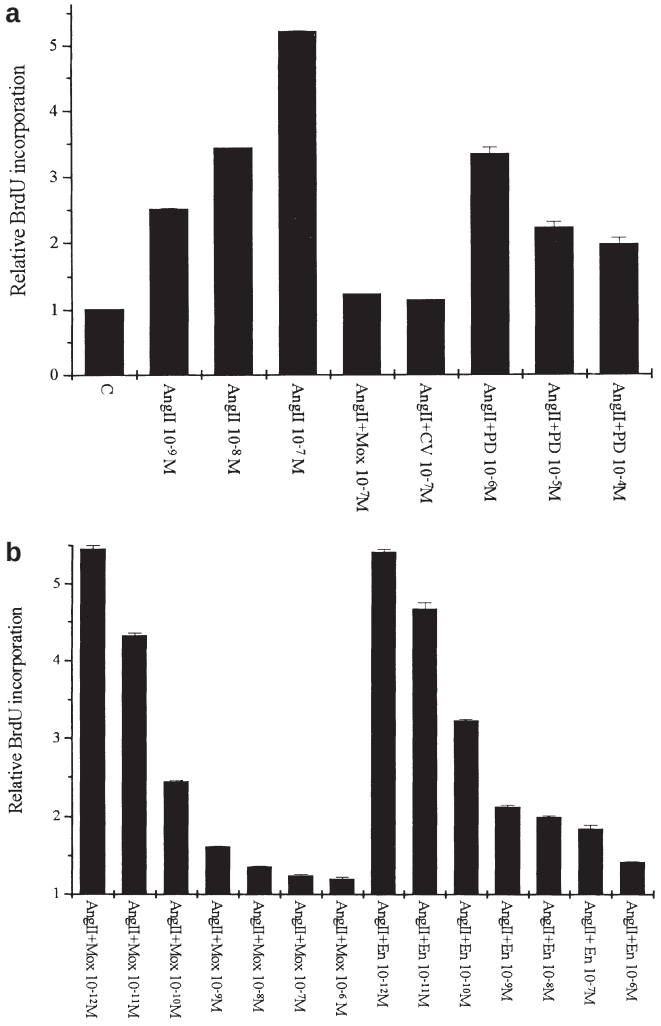

Fig. 1 **a** Influence of Ang II on rat cardiac fibroblast proliferation as measured by BrdU incorporation. Cells were grown in the absence or presence of Ang II (10^{-9}–10^{-7} M; *AngII*) and coincubated with either the ACE inhibitor moexiprilat (10^{-7} M; *Mox*), the AT_1 receptor antagonist candesartan (10^{-7} M; *CV*), the MEK1 inhibitor PD 98059 (10^{-6}–10^{-4} M; *PD*), respectively, for 24 h. *Bars* represent the DNA synthesis as measured by BrdU incorporation with SEM. Activity is shown relative to cells treated with vehicle alone (*C*). Ang II led to a concentration-dependent significant increase of DNA synthesis compared to cells treated with vehicle alone. While moexiprilat (10^{-7} M) and candesartan (10^{-7} M) led to a complete regression of Ang II (10^{-7} M) induced growth, PD98059 only led to partial inhibition of DNA synthesis resembling a 45%, 72% and 77% reduction in comparison to Ang II (10^{-7} M) stimulated cells. In unstimulated cells, the antagonists had no significant effect on BrdU incorporation. All values consist of an $n = 9$. **b** Comparison of the antiproliferative effect of moexiprilat and enalaprilat

0.09-fold; $n = 9$; Fig. 1a). The effect was completely blocked by coincubation with the AT_1 receptor antagonist CV11974 (candesartan; 10^{-7} M), indicating that this process is mediated via the AT_1 receptor (Ang II + CV: 1.14 ± 0.01-fold). Interestingly, the ACE inhibitor moexiprilat (10^{-12}–10^{-6} M) also led to a dose-dependent inhibition of the Ang II (10^{-9}–10^{-6} M) stimulated growth (data for AngII 10^{-7} M; Ang II 10^{-9} M, 10^{-8} M and 10^{-6} M revealed a similar dose response curve; $n = 9$; Fig. 1b). In control experiments moexiprilat did not inhibit FCS (1% or 10%) induced growth, demonstrating the specificity for Ang II mediated growth and a lack of unspecific toxicity (data not shown). To further elucidate if the observed effect is mediated via ACE inhibition, the potency of moexiprilat was compared to that of enalaprilat. The ACE inhibitor enalaprilat (10^{-12}–10^{-6} M caused a dose-dependent decrease in Ang II (10^{-9}–10^{-6} M) stimulated growth (data for AngII 10^{-7} M, Ang II 10^{-6} M, 10^{-8} M and 10^{-9} M revealed a similar dose-response curve; $n = 9$; Fig. 1b). In comparison, both substances led to a dose-dependent decrease in proliferation with enalaprilat being less potent than moexiprilat in inhibiting the BrdU incorporation (Ang II + Mox 10^{-12} M: 5.54 ± 0.13-fold; Ang II + Mox 10^{-11} M: 4.33 ± 0.09-fold; Ang II + Mox 10^{-10} M: 2.44 ± 0.06-fold; Ang II + Mox 10^{-9} M: 1.66 ± 0.03-fold; Ang II + Mox 10^{-8} M: 1.36 ± 0.04-fold; Ang II + Mox 10^{-7} M: 1.24 ± 0.05-fold; Ang II + Mox 10^{-6} M: 1.20 ± 0.07-fold; Ang II + En 10^{-12} M: 5.41 ± 0.14-fold; Ang II + En 10^{-11} M: 4.67 ± 0.24-fold; Ang II + En 10^{-10} M: 3.23 ± 0.06-fold; Ang II + En 10^{-9} M: 2.12 ± 0.07-fold; Ang II + En 10^{-8} M: 1.99 ± 0.06-fold; Ang II + En 10^{-7} M: 1.84 ± 0.14-fold; Ang II + En 10^{-6} M: 1.41 ± 0.04-fold). Angiotensin III (Ang III; 10^{-7} M), a breakdown product of Ang II, had a minimal effect on cardiac fibroblast proliferation (1.62 ± 0.12-fold increase in BrdU incorporation; data not shown).

Recent studies have shown that Ang II also activates the cytokine and growth factor related JAK/STAT signalling pathway (Bhat et al. 1994). However, no specific inhibitor of this signalling pathway is currently known. To further elucidate the mechanisms of the mitogenic response in Ang II stimulated cardiac fibroblasts, we examined critical signalling pathways such as the MAPKs and the JAK/STAT pathway.

Ang II (10^{-7} M) led to activation of the MAPKs pathway, measured by phosphorylation of ERK1/2 in immunoblot analysis using a phospho-specific antibody directed against pERK1/2. Coincubation with the AT_1 receptor antagonist candesartan (10^{-7} M) led to a complete inhibition of the Ang II (10^{-7} M) induced ERK1/2 phosphorylation, indicating that this process is mediated via the AT_1 receptor (Fig. 2a, c). Coincubation with the ACE inhibitor moexiprilat (10^{-7} M) also led to a complete inhibition of the ERK1/2 phosphorylation by Ang II (10^{-7} M; Fig. 2a, c). Activation of G-protein coupled receptors leads to a phosphorylation of p38-MAPK, another member of the MAPK family in cardiac myocytes (Sugden and Clerk 1998). In cardiac fibroblasts, Ang II (10^{-7} M) also caused an induction of p38-MAPK phosphorylation as shown by immunoprecipitation with subsequent detection of phosphorylated tryosine residues of p38-MAPK. This phosphorylation could be completely inhibited by coincubation with moexiprilat (10^{-7} M) or enalaprilat (10^{-7} M; Fig. 2b, c). Statistical analysis of the densitometrical data of three independent immunoblots revealed the following induction of ERK1/2 and p38-MAPK phosphorylation in comparsion to cells treated with vehicle alone: ERK1/2 – Ang II: 1.71 ± 0.02-fold; Ang II + Mox: 0.96 ± 0.01-fold;

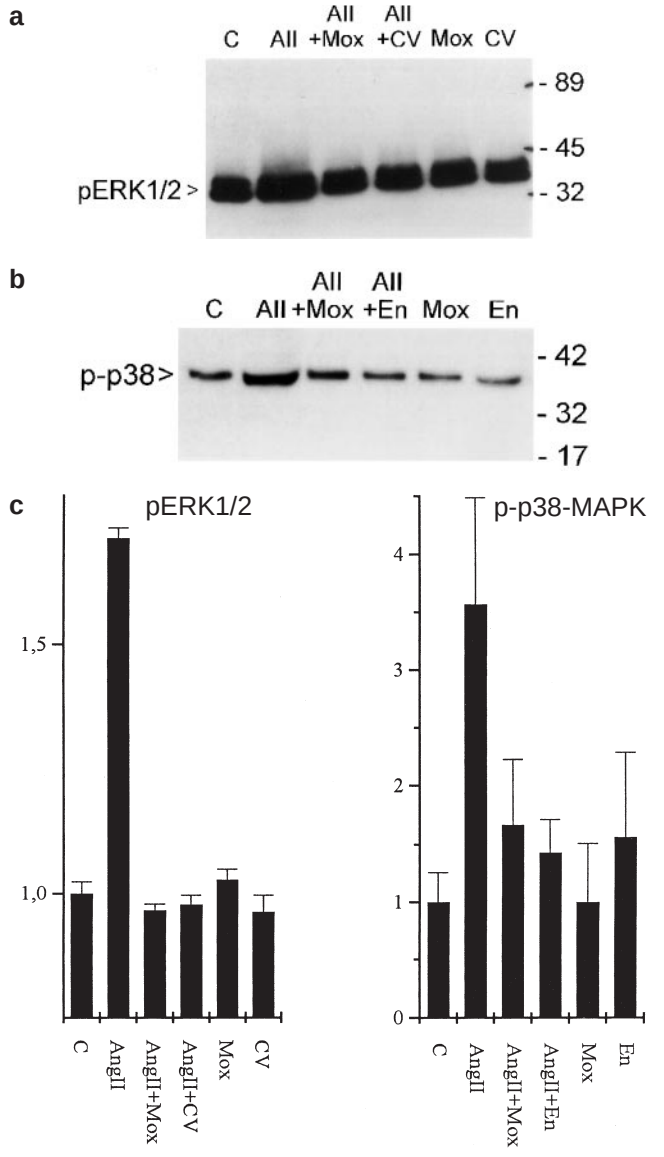

Fig. 2 **a** Ang II (10^{-7} M) led to a time-dependent increase in MAPK activity, measured by an immunoblot using a phospho-specific pERK1/2 antibody, with a maximum increase between 5 min and 15 min. Ang II (10^{-7} M, 5 min; *AII*) induced phosphorylation of ERK1/2 was completely inhibited by a coincubation with the ACE inhibitor moexiprilat (10^{-7} M, 5 min; *AII + Mox*) or a coincubation with the AT_1 receptor antagonist candesartan (10^{-7} M, 5 min; *AII + CV*). Moexiprilat (10^{-7} M, 5 min) alone (*Mox*) and candesartan alone (10^{-7} M, 5 min; *CV*) had no significant effect on basal ERK1/2 phosphorylation. Control cells were treated with vehicle alone (*C*). One of three similar studies is shown. **b** Ang II (10^{-7} M, 60 min) also led to the phosphorylation of p38-MAPK (p-p38). This effect was inhibited by a coincubation with either moexiprilat (10^{-7} M, 60 min; *AII + Mox*) or enalaprilat (10^{-7} M, 60 min; *AII + En*). The ACE inhibitors alone (10^{-7} M, 60 min) had no effect on basal p38-MAPK phosphorylation (*Mox, En*). One of three similar studies is shown. **c** Densitometrical analysis of three immunoblots in comparison to cells treated with vehicle alone

Ang II + CV: 0.98 ± 0.02-fold; Mox: 1.02 ± 0.02-fold; CV: 0.96 ± 0.3-fold and p38-MAPK – Ang II: 3.57 ± 0.92-fold; Ang II + Mox: 1.67 ± 0.56-fold; Ang II + En: 1.43 ± 0.29-fold; Mox: 1.00 ± 0.51-fold; En: 1.56 ± 0.72-fold.

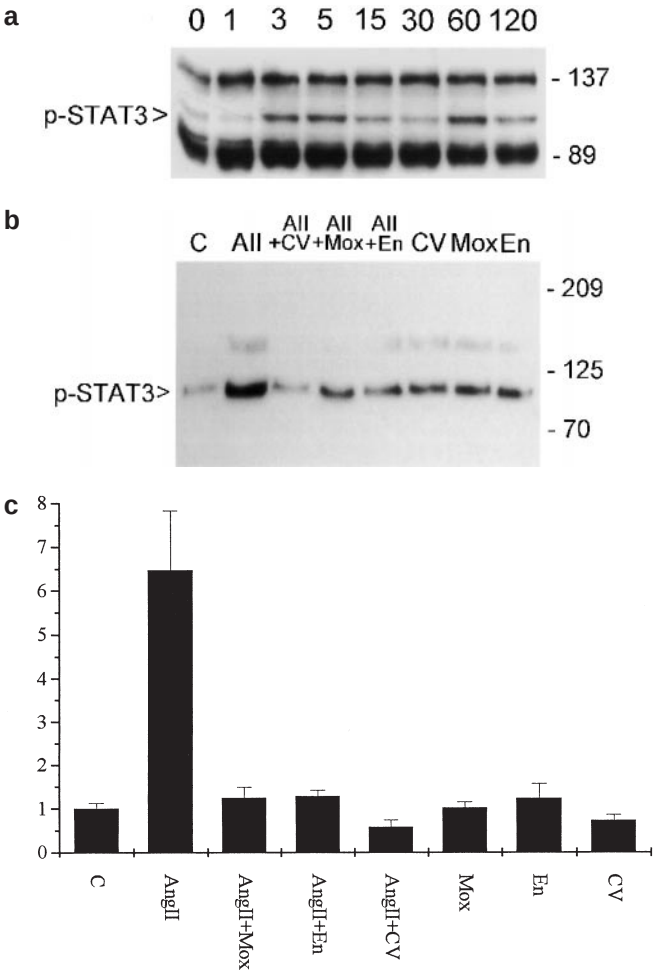

Fig. 3 **a** Immunoblot analysis with a phospho-specific pSTAT3 antibody revealed, that Ang II (10^{-7} M) stimulation led to a time-dependent increase in STAT3 phosphorylation with a maximum at 5 min and at 60 min. **b** Ang II (10^{-7} M, 60 min; *AII*) induced phophorylation of STAT3 was completely inhibited by coincubation with the ACE inhibitors moexiprilat (10^{-7} M, 60 min; *AII + Mox*) and enalaprilat (10^{-7} M, 60 min; *AII + En*) or a coincubation with the AT_1 receptor antagonist candesartan (10^{-7} M, 60 min; *AII + CV*). Moexiprilat (10^{-7} M, 60 min; *Mox*) and enalaprilat (10^{-7} M, 60 min; *En*) alone had no significant effect on basal STAT3 phosphorylation, while candesartan alone (10^{-7} M, 60 min) suppressed basal STAT3 phosphorylation significantly (*CV*). Control cells were treated with vehicle alone (*C*). One of three similar studies is shown. **c** Densitometrical analysis of three immunoblots in comparison to cells treated with vehicle alone

To study another pivotal mitogenic pathway, we examined the cytokine and growth-factor related JAK/STAT pathway. Ang II (10^{-7} M) led to a time-dependent activation of STAT3, measured by the phosphorylation of STAT3 in immunoblot analysis using a phospho-specific antibody directed against pSTAT3 in neonatal rat cardiac fibroblasts (Fig. 3 a). The two unspecific bands observed in blot a were not detectable after a reduction of the antibody concentration to 1 : 500. This effect was mediated via the AT_1 receptor, and a coincubation with the AT_1 receptor antagonist candesartan (10^{-7} M) caused a complete inhibition of the Ang II (10^{-7} M) induced STAT3 activa-

tion (Fig. 3b, c). Interestingly, coincubation with the ACE inhibitors moexiprilat (10^{-7} M) or enalaprilat (10^{-7} M) also led to a complete inhibition of the phosphorylation of STAT3 by Ang II (10^{-7} M) as shown in Fig. 3b, c. Statistical analysis of the densitometrical data of three independent immunoblots revealed the following induction of STAT3 phosphorylation in comparsion to cells treated with vehicle alone: Ang II: 6.47 ± 1.36-fold; Ang II + Mox: 1.24 ± 0.25-fold; Ang II + En: 1.28 ± 0.13-fold; Ang II + CV: 0.56 ± 0.18-fold; Mox: 1.00 ± 0.15-fold; En: 1.22 ± 0.34-fold; CV: 0.72 ± 0.14-fold.

Discussion

Cardiac fibroblasts play a major role in the progression of left ventricular hypertrophy and cardiac remodeling (Brooks et al. 1997; Schieffer et al. 1994; Zhu et al. 1997). Studies have identified ACE (Dostal et al. 1992) and AT_1 receptors (Villarreal et al. 1993) on cardiac fibroblasts. Ang II can exert mitogenic effects on cardiac fibroblasts via several signal transduction pathways including the MAPKs and the JAK/STAT pathway (Bhat et al. 1994; Schorb et al. 1995; Zou et al. 1998). All of these effects are mediated via the AT_1 receptor (Regitz-Zagrosek et al. 1996). As our group reported earlier, coincubation with the ACE inhibitor moexiprilat leads to an inhibition of Ang II induced immediate early gene (c-fos, egr-1 and sp-1) expression (Grohé et al. 1998). Furthermore, we have demonstrated previously that moexiprilat inhibits cardiac fibroblast growth induced by estrone, the predominant estrogen in postmenopausal women (Grohé et al. 1997). To further elucidate growth related signalling pathways involved in this process, we examined the influence of Ang II on the activation of the MAPKs and the JAK/STAT pathways and the effects of ACE inhibition on this process. The activation of ERK1/2 is critical for the enhancement of Ang II induced DNA synthesis in cardiac fibroblasts (Zou et al. 1998). Bhat et al. (1994) have shown that Ang II leads to an activation of the JAK/STAT pathway, by demonstrating the increased activity of the sis inducing factor SIF in cardiac fibroblasts. The SIF is a homo- or heterodimer of the phosphorylated forms of STAT1 and STAT3 (Bhat et al. 1995). Here we demonstrated that Ang II leads to a time-dependent phosphorylation of STAT3 in cardiac fibroblasts. This process is likely to be mediated via the AT_1 receptor since it was completely inhibited by coincubation with an AT_1 receptor antagonist. This is consistent with the effects observed by Bhat et al. (1995) in T3CHO/AT_{1A} cells, a cell line transfected with the AT_1 receptor.

In conclusion, Ang II leads to a dose-dependent activation of cardiac fibroblast growth. This process is inhibited by the coincubation with the ACE inhibitors moexiprilat and enalaprilat. The reduced potency of enalaprilat in comparison to moexiprilat closely matches the relative potencies of these substances in the inhibition of ACE activity in vitro. This observation suggests that the antiproliferative effect described in this paper is mediated via the inhibition of ACE. However, it is noteworthy that the absolute potencies of moexiprilat and enalaprilat are 2 log steps lower in our model of cultured cardiac fibroblasts compared to experiments using ACE obtained from tissue homogenates or serum (Edling et al. 1995). There is only a minor effect of Ang III on cardiac fibroblast growth, therefore this breakdown product of Ang II is not responsible for the observed effect.

Since ACE inhibition leads to a complete inhibition of the Ang II induced phosphorylation of ERK1/2, p38-MAPK and STAT3, these signalling pathways may be involved in the antiproliferative effects of ACE inhibitors on Ang II stimulated cardiac fibroblast growth. Interestingly, the ACE inhibitors moexiprilat and enalaprilat show similar effects as the AT_1 receptor antagonist candesartan. These data suggest that ACE inhibitors not only block the conversion of Ang I into Ang II, but also influence Ang II mediated effects on the heart. However, further studies are necessary to reveal the mechanisms by which ACE inhibitors modulate mitogenic signalling pathways in the process of cardiac hypertrophy.

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