Differential effects of 17β-estradiol on mitogen-activated protein kinase pathways in rat cardiomyocytes

Simone Nuedling[a], Stefan Kahlert[a], Kerstin Loebbert[a], Rainer Meyer[b], Hans Vetter[a], Christian Grohé[a,*]

[a] *Medizinische Poliklinik, University of Bonn, Wilhelmsstr. 35–37, 53111 Bonn, Germany*
[b] *Institut für Physiologie II, University of Bonn, 53111 Bonn, Germany*

Received 4 June 1999

Abstract Cardiac myocytes contain functional estrogen receptors, however, the effect of estrogen on growth-related signaling pathways such as mitogen-activated protein kinases (MAPK) in the pathogenesis of cardiac disease is unclear. MAPKs are critically involved in regulatory signaling pathways which ultimately lead to cardiac hypertrophy. Here we show that 17β-estradiol (E2) activates extracellular signal-regulated kinase (ERK1/2), c-Jun-NH_2-terminal protein kinase (JNK) and p38 in rat cardiomyocytes in a distinctive pattern. As shown by immunoblot analysis and phosphorylation assays, E2 (10^{-9} M) induced a rapid and transient activation of ERK1/2 and a rapid but sustained increase of JNK phosphorylation. In contrast, E2 had only a marginal effect on p38 activation. Furthermore, MAPK phosphatase expression was induced by E2 and E2-stimulated expression of endothelial and inducible NO synthase was inhibited by PD 98059, an inhibitor of the ERK pathway. These novel observations may help to explain the role of estrogen in gender-based differences found in cardiac disease.

Key words: Extracellular signal-regulated kinase; p38; 17β-Estradiol; c-Jun-NH_2-terminal protein kinase; Cardiomyocyte

1. Introduction

Cardiac diseases such as left ventricular hypertrophy reveal significant gender-based differences [1,2]. Left ventricular hypertrophy is an important risk factor for cardiovascular disease and is associated with an increased risk of arrhythmias and sudden death [3]. Estrogen has been suggested to play a major role in the pathogenesis of this process as the incidence in women increases after the onset of menopause [4]. Estrogen is a member of a large family of steroid hormones that regulate gene transcription in estrogenic target tissues upon binding to the respective intracellular estrogen receptor (ER) [5,6]. To date, two different estrogen receptors, ERα and ERβ, have been identified and characterized [7,8]. The recently cloned ERβ is highly homologous to the 'classical' ERα and has been shown to bind estrogens with an affinity similar to ERα, and activates expression of genes containing estrogen response elements [9]. Cardiac myocytes contain functional estrogen receptors of both subtypes that regulate expression of target genes such as NO synthases [10,11]. In addition, there are important rapid, therefore termed non-genomic, effects of estrogens on the myocardium [12,13]. These non-genomic effects of estrogen in cardiac myocytes are poorly understood but may play an important role in the pathogenesis of cardiac hypertrophy and cardiac remodeling after myocardial infarction. To elucidate the effect of estrogen on signal transduction pathways involved in the pathogenesis of cardiac disease we investigated the influence of 17β-estradiol on different mitogen-activated protein kinases (MAPKs) in cardiomyocytes. MAPKs have been identified as a family of protein Ser/Thr-kinases that are rapidly activated by dual phosphorylation of their Tyr-Xaa-Thr motif. This phosphorylation is catalyzed by dual-specific MAPK kinases (MKK/MEK) in response to a wide array of extracellular stimuli [14]. Well characterized subfamilies of the MAPK superfamily are the extracellular signal-regulated kinases (ERKs) and the two stress-responsive MAPK subfamilies, the c-Jun-NH_2-terminal protein kinase/stress-activated protein kinase (JNK/SAPK) and the p38 MAPK [15–17]. Different cell stimuli appear to activate distinct MAPKs preferentially. While growth factors and some oncogenes are linked to activation of ERK, inflammatory cytokines and cell stress lead to activation of JNK/SAPK and p38 [18]. Here we examined the effect of estrogen on MAPK families as mediators of estrogenic effects in adult and neonatal rat cardiomyocytes. Inactivation of MAPK is mediated, in part, by dephosphorylation of MAPK by dual-specificity phosphatases called MAPK phosphatases (MKP) that dephosphorylate both threonine and tyrosine residues phosphorylated by MEK [19,20]. Since MKP is principally regulated at the transcriptional level, we studied the MKP1 gene expression in response to estrogen. Finally, we examined the influence of MAPK phosphorylation on estrogen-responsive cardiac genes such as the endothelial and inducible NO synthase as cardiac NO production through NO synthases has been suggested to play a crucial role in the regulation of cardiac muscle function [21,22].

2. Materials and methods

2.1. Materials

All chemicals were obtained from Merck (Darmstadt, Germany) if not otherwise specified.

2.2. Isolation and culture of adult cardiomyocytes

Procedures with experimental animals followed the guidelines of the National Institute of Environmental Health Sciences Animal and Use Committee. Cardiomyocytes were isolated after a modified protocol by Claycomb and Palazzo [23] from adult female Wistar-Kyoto rats weighing 200–350 g. Animals were anesthetized with diethylether and heparinized with 2500 units sodium heparin into the vena cava. The heart was rapidly dissected and mounted on the cannula of a Langendorff perfusion system. Retrograde perfusion was performed ac-

*Corresponding author. Fax: (49) (228) 287-2266.
E-mail: c.grohe@uni-bonn.de

PII: S0014-5793(99)00816-9

cording to the procedure of Powell [24] using a calcium-free perfusion buffer containing 128 mM NaCl, 14 mM KCl, 190 μM NaH_2PO_4, 1 mM Na_2HPO_4, 1.5 mM $MgSO_4$, 10 mM HEPES, 5.5 mM D-glucose, 2 mM sodium pyruvate. Enzyme solution consisted of 1.3 mg/ml collagenase (0.452 U/mg, Boehringer Mannheim, Germany) and 5 mg/ml albumin, fraction V, fatty acid-free (Boehringer Mannheim). Thereafter, total ventricular tissue was chopped, minced into small pieces, incubated in 2% albumin solution (5 min) and passed through a mesh of 250 μm pore size. Cardiac fibroblasts and non-myocytes were separated from cardiac myocytes by three consecutive 2% serum albumin gradient steps. Freshly isolated cardiomyocytes were counted in a Fuchs-Rosenthal chamber and contained 80–85% rod-shaped ventricular myocytes. Cells were seeded on culture dishes at a density of 2×10^4 cells/cm^2 and cultured in medium 199 (Gibco, Eggenstein, Germany) supplemented with 2 mM carnitine, 5 mM taurine, 5 mM creatine, penicillin (100 U/ml) and streptomycin (100 U/ml) (CCT199 medium, pH 7.4).

2.3. Isolation and culture of neonatal cardiomyocytes

The hearts of 1–2 day old rats 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 as previously described [10]. Cell suspension was centrifuged at $400\times g$ for 5 min and the cell pellet was resuspended in 20 ml of Ham's F10 supplemented with 10% horse serum (Biochrom, Berlin, Germany) and 10% estrogen-free fetal calf serum (FCS; c.c.pro, Neustadt, Germany) and plated on culture dishes. After 75 min the medium which contained the cardiomyocyte fraction of the digested tissue was removed. Cardiomyocytes were counted in a Fuchs-Rosenthal chamber and seeded on culture dishes at a density of 2×10^4 cells/cm^2. The neonatal cardiomyocyte culture contained 90–95% myocytes, as assessed by immunofluorescence staining with an antibody against troponin-t (CP05, Dianova, Hamburg, Germany).

2.4. Treatment of cardiomyocytes

Serum-starved cells were treated with 10^{-9} M 17β-estradiol in a time-dependent fashion for 5, 15, 30, 60, or 120 min with or without 10^{-8} M ICI 182.780 (kind gift of Dr. A.E. Wakeling), a specific pure antiestrogen [25]. Control cells were incubated with 0.1% (v/v) ethanol, the solvent of 17β-estradiol. Additional controls were coincubated with 10^{-5} M PD 98059 (Calbiochem, Bad Soden, Germany), a selective inhibitor of MAPK kinase (MEK1/2), which catalyzes the phosphorylation of MAPK [26], and with SB 203580 (10^{-5} M), a highly specific inhibitor of p38 kinase [27]. Moreover, a series of controls was performed with the stereoisomer 17α-estradiol, known to be biologically ineffective as an estrogen. After stimulation cells were rinsed with phosphate-buffered saline and lysed with 500 μl lysate buffer (50 mM NaCl, 50 mM NaF, 20 mM Tris, 10 mM EDTA, 20 mM $Na_4P_2O_7$, 1 mM Na_3VO_4, 1% Triton X-100, 1 mM PMSF, 10 μg/ml antipain, 10 μg/ml leupeptin, 10 μg/ml aprotinin). Lysates were clarified by centrifugation at $12\,000\times g$ for 10 min at 4°C. Protein content was measured with a standard Bradford assay. For a positive control we include 20 ng active MAPK (ERK2, New England Biolabs, Schwalbach, Germany) to 200 μl of control cell extract.

2.5. Western blot analysis

Total cell lysates (40 μg/lane) of each sample were subjected to SDS-PAGE on 7.5% gels for resolution of ERK, JNK and p38 MAPK. Protein was transferred electrophoretically to a nitrocellulose membrane. Equal transfer among lanes was verified by reversible staining with Ponceau red. Immunoblotting was performed with a monoclonal dual phospho-specific (threonine-202/tyrosine-204) antibody directed against the activated ERK1/2 (1:1000; New England Biolabs) or a polyclonal phospho-specific antibody against JNK (1:500; Santa Cruz, Heidelberg, Germany) or a polyclonal dual phospho-specific antibody against p38-MAPK (1:500; New England Biolabs). In order to investigate gene expression of MKP1 as well as eNOS- and iNOS-specific antibodies against MKP1 (1:500; Santa Cruz), iNOS (1:500; Calbiochem) and eNOS (1:500; Calbiochem) were used. Detection was performed with the enhanced chemiluminescence technique (ECL; Amersham, Braunschweig, Germany). Densitometric analysis of immunoblots was performed on an Epson GT 8000 scanner with the analysis software ScanPak (Biometra, Göttingen, Germany).

2.6. MAPK assay

In order to determine the activation potential of different MAPKs induced by estrogen, we used specific MAPK phosphorylation assays (p42/p44 MAPK Assay Kit, JNK/SAPK Assay Kit, p38 MAPK Assay Kit; New England Biolabs). Briefly, a monoclonal dual phospho-specific antibody directed against the phosphorylated ERK or p38 protein was used to selectively immunoprecipitate active ERK1/2 and p38 from total cell lysates. To analyze MAPK activity the resulting immunoprecipitate was incubated in the presence of ATP and kinase buffer with an Elk1 (Ets [E26 transformation-specific]-like protein 1) fusion protein as a substrate for ERK and with ATF-2 fusion protein as a substrate for p38. Phosphorylation of the respective substrate was determined by Western blot analysis using phospho-specific antibodies against Elk1 or ATF-2. JNK assay was performed using c-Jun fusion protein beads containing high affinity binding sites for JNK. After binding of JNK to c-Jun fusion protein kinase reaction was carried out using c-Jun as substrate. Phosphorylation of c-Jun was analyzed by Western blot analysis using a phospho-specific antibody against c-Jun protein.

2.7. Statistical analysis

All experimental data consist of a minimum of at least three independent experiments from three different preparations. All reported values are mean ± S.E.M. Statistical comparisons were made by Student's *t*-test. Statistical significance was assumed if a null hypothesis could be rejected at the $P < 0.05$ level.

3. Results

To investigate non-genomic effects of estrogen on cardiac myocytes we examined the influence of estrogen on different MAPKs, namely ERK1/2, the JNK/SAPK and p38. Immunoblot analyses with phospho-specific antibodies selectively directed against the activated, phosphorylated MAPK proteins were performed. Total cell lysates of cardiac myocytes derived from cardiac myocytes from adult female rats (Fig. 1A) and from neonatal rats (Fig. 1B) revealed only a low level of activated 42 kDa and 44 kDa isoforms of ERK1 and ERK2 in the absence of 17β-estradiol (E2). Stimulation of cardiac myocytes with E2 (10^{-9} M) for 5–120 min induced a rapid and transient increase of phosphorylated ERK1/2 protein. ERK phosphorylation in adult female cardiomyocytes reached a maximum of 3.3 ± 0.7-fold after 5 min incubation (Fig. 1C). The ERK phosphorylation pattern in lysates obtained in neonatal cardiomyocytes displayed a similar pattern and maximal activation occurred after 15 min incubation. Cotreatment with the pure ER-specific antagonist ICI 182.780 (10^{-8} M) completely negated the rapid E2-stimulated increase in ERK phosphorylation at 15 min incubation.

To further elucidate the effect of E2 on ERK phosphorylation in cardiac myocytes we studied the phosphorylation of ERK substrates such as Elk1, which was determined by an ERK phosphorylation assay. Incubation with E2 (10^{-9} M) increased phosphorylation of the target protein Elk1 by ERK1/2 in cardiomyocytes obtained from adult female rats (Fig. 2A) as well as from neonatal rats (Fig. 2B). E2 induced a rapid and transient phosphorylation of ERK target protein demonstrating that ERK1/2 phosphorylation paralleled ERK1/2 activity. Maximum induction of ERK1/2 activity occurred at 15 min (2.3 ± 0.2-fold). Cotreatment of cardiomyocytes with the ER-specific antagonist ICI 182.780 (10^{-8} M) and with a specific MAPK kinase blocker, PD 98059 (10^{-5} M) inhibited the E2-mediated phosphorylation of the MAPK protein and in consequence the phosphorylation of Elk1.

To extend our previous observations we studied the effect of E2 on additional MAPK signaling pathways, namely the JNK and p38 pathways. JNK activity was determined by immunoblot analyses of total cell lysates with an antibody that specifically recognizes the phosphorylated form of JNK. In the absence of E2, the level of JNK phosphorylation in cardiomyocytes from adult female (Fig. 3A) and neonatal rats (Fig. 3B) was low. After stimulation with 10^{-9} M E2 JNK phosphorylation increased rapidly and was sustained over time. Cotreatment of cardiomyocytes with the specific ER antagonist ICI 182.780 (10^{-8} M) completely inhibited phosphorylation of JNK. These results were confirmed using a JNK assay that specifically measured JNK-induced phosphorylation of c-Jun. Incubation with E2 (10^{-9} M) increased phosphorylation of the JNK substrate c-Jun in a time-dependent fashion (Fig. 3C) and revealed a pattern that showed that JNK phosphorylation paralleled JNK activity.

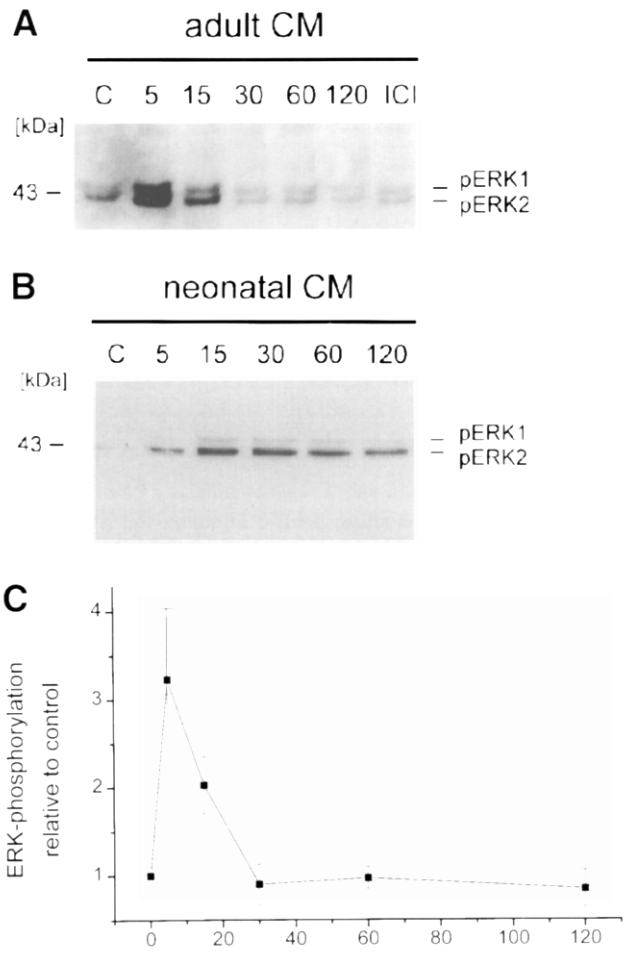

Fig. 1. Time-dependent activation of ERK1/2 by E2. Cardiomyocytes from adult female rats (A) and from neonatal rats (B) were stimulated with 17β-estradiol (E2, 10^{-9} M) for the indicated time periods (5–120 min). Cell lysates were subjected to SDS-PAGE, immunoblotted with an antibody specifically detecting the phosphorylated ERK1/2, and visualized by a chemiluminescence technique. Note that the phosphorylated protein (pERK) is detectable in the absence of 17β-estradiol (Control, C), but is more abundant following treatment with E2 in a time-dependent fashion. Coincubation with the antiestrogen ICI 182.780 (ICI, 10^{-8} M) for 15 min inhibited the phosphorylation. C: Time course of E2-induced phosphorylation of ERK1/2 in adult rat cardiomyocytes. Relative kinase phosphorylation was determined by densitometric analysis of immunoblots. The results are expressed as stimulated kinase activity divided by unstimulated activity and are mean ± S.E.M. from three experiments.

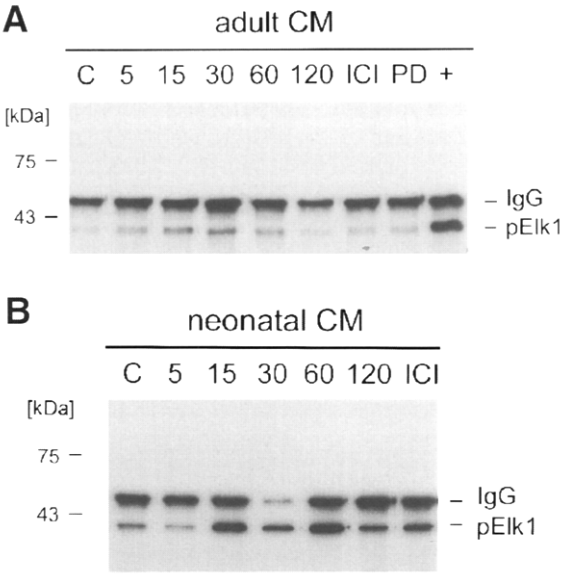

Fig. 2. Time-dependent activation of the ERK1/2 substrate Elk1 by E2. ERK1/2 phosphorylation assays show induction of Elk1 phosphorylation by E2 in a time-dependent fashion. A monoclonal dual phospho-specific antibody was used to immunoprecipitate active ERK1/2 from total cellular lysates of adult female (A) and neonatal (B) cardiomyocytes incubated in the absence (C) or presence (5–120 min) of E2 (10^{-9} M). The resulting immunoprecipitate was incubated with an Elk1 fusion protein. Phosphorylation of Elk1 was analyzed by Western blot analysis using a phospho-Elk1 antibody. Phosphorylated Elk1 protein is detectable in the absence of E2, but increases in a time-dependent fashion following E2 treatment. Coincubation with PD 98059 (PD, 10^{-5} M) and ICI 182.780 (ICI, 10^{-8} M) for 15 min inhibited ERK1/2-mediated phosphorylation of Elk1. Phosphorylated ERK1/2 (+) served as a positive control. Phosphorylation levels were normalized to IgG to correct for differences in protein loading.

Furthermore, we investigated the effect of E2 on p38 phosphorylation. In contrast, stimulation with E2 did not significantly influence p38 phosphorylation in cardiomyocytes from adult female rats as shown by Western blot analysis (Fig. 4A) and phosphorylation assay (Fig. 4B). Cotreatment with the ER antagonist ICI 182.780 (10^{-8} M) had no effect on p38 phosphorylation and activation, but cotreatment with the specific inhibitor of p38, SB 203580 (10^{-5} M), decreased activation of p38.

The response was specific to E2, because 17α-estradiol (10^{-9} M), known to be biologically ineffective as an estrogen, failed to alter the activation of ERK, JNK and p38 phosphorylation (data not shown). All experiments were repeated with lysates obtained from adult male rats revealing a similar effect of E2 on these signaling cascades which demonstrates a gender-independent phenomenon (data not shown).

Termination of MAPK activity is regulated by MKP, which dephosphorylates and inactivates MAPK. The expression level of MKP1 protein correlates with the kinetics of dephosphorylation of MAPK. Since MKP1 is principally regulated at the transcriptional level, we studied MKP1 gene expression in cardiomyocytes in response to stimulation with E2 (Fig. 5). MKP1 protein expression increased after 30 min stimulation with E2 and correlated with the kinetics of ERK1/2 dephos-

phorylation. MKP1 expression was completely inhibited by cotreatment with the ER antagonist ICI 182.780 (10^{-8} M).

Finally, we examined if estrogenic target genes such as the endothelial and inducible NO synthases (eNOS/iNOS) are regulated by estrogen via the MAPK pathway. Treatment of cardiomyocytes with E2 for 60 min (10^{-9} M) causes an increase of NOS expression (iNOS: 23.4 ± 9.1-fold; eNOS: 6.0 ± 1.6-fold). Coincubation with an inhibitor of the MAPK pathway, PD 98059, suppressed the upregulation of iNOS (Fig. 6A) and eNOS (Fig. 6B) in cardiomyocytes. These results suggest that eNOS and iNOS expression induced by E2 is at least partially dependent on MAPK phosphorylation.

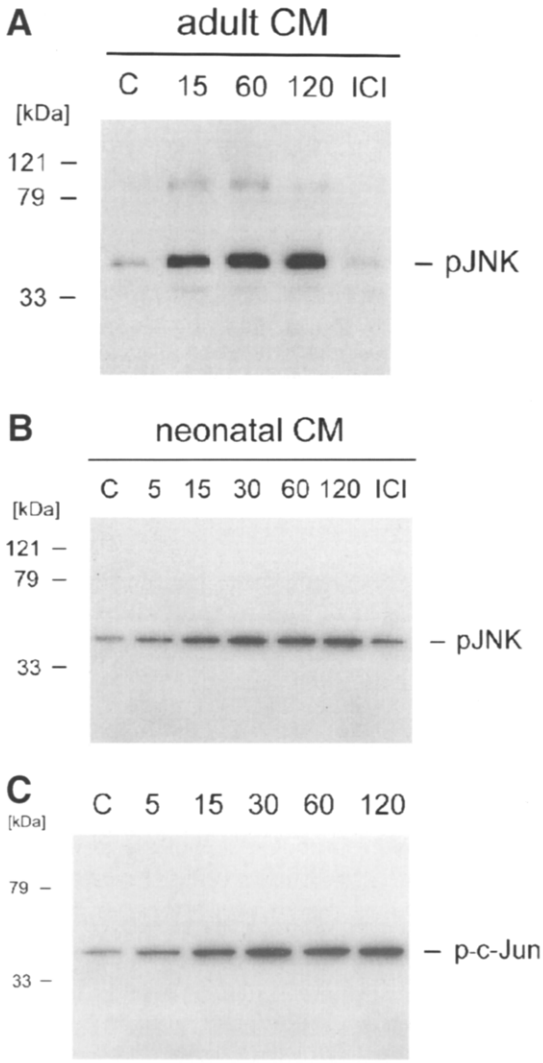

Fig. 3. Induction of JNK phosphorylation in cardiomyocytes by E2. Cardiomyocytes obtained from adult female (A) and neonatal (B) cardiomyocytes were treated with E2 (10^{-9} M) for the indicated time periods (5–120 min). Total cellular lysates were subjected to SDS-PAGE, and immunoblotted with an antibody specifically detecting the phosphorylated JNK. Note that the phosphorylated protein (pJNK) is detectable in the absence of E2 (Control, C), but is more abundant following treatment with E2. Coincubation with the antiestrogen ICI 182.780 (10^{-8} M, 15 min) inhibited the E2-mediated phosphorylation of JNK. C: JNK phosphorylation assay shows activation of JNK target protein c-Jun in a time-dependent fashion which paralleled JNK phosphorylation.

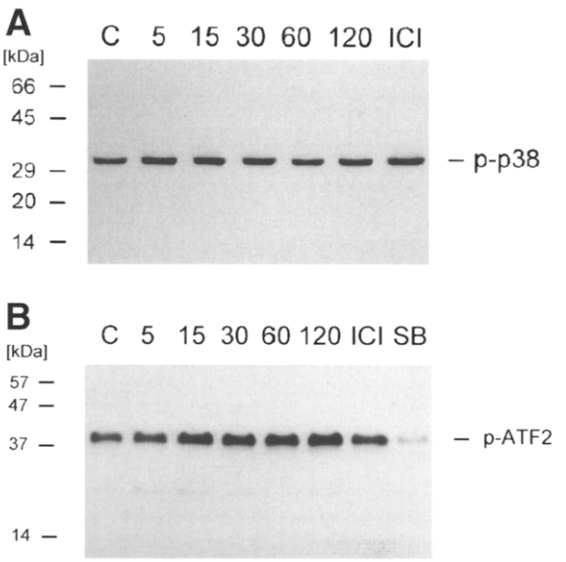

Fig. 4. p38 activity stimulated by E2. Adult female cardiomyocytes were stimulated with E2 (10^{-9} M) for the indicated time periods (5–120 min). Control cells were coincubated with the specific ER antagonist ICI 182.780 (ICI, 10^{-8} M, 15 min) and with an specific inhibitor of p38, SB 203580 (SB, 10^{-5} M, 15 min). A: Phosphorylation level of p38 was examined by Western blot analysis using an antibody specifically detecting the phosphorylated p38 protein. E2 did not cause a significant alteration of p38 phosphorylation. B: Influence of E2 on p38-mediated phosphorylation of ATF2 as shown by phosphorylation assay.

4. Discussion

The role of estrogen in the pathogenesis of gender-based differences found in cardiac disease is currently under investigation [1–4,28]. The importance of hormonal regulation of cardiac disease is underlined by the observation that women tend to develop cardiac hypertrophy only after the onset of menopause. All the clinical sequelae of coronary artery disease are worsened in patients with left ventricular hypertrophy in coincidence with an six-fold increase in sudden death [29]. Up to now most cellular effects of estrogen in the myocardium have been thought to be mediated by intracellular estrogen receptors that serve as ligand-induced transcription factors. In cardiomyocytes estrogen activates downstream target genes such as connexin 43 or NO synthases via ERα and β [10,11]. Furthermore, rapid, therefore termed non-genomic, actions of

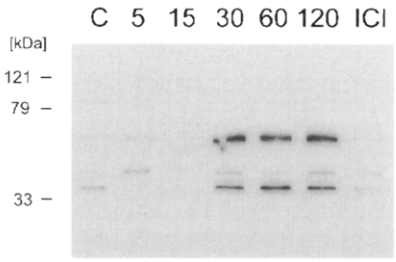

Fig. 5. Expression of MKP1 in cardiomyocytes is induced by E2. Cardiomyocytes from neonatal rats were stimulated with E2 (10^{-9} M) for the indicated time periods (5–120 min). Cell lysates were analyzed by Western blot analysis using an antibody against MKP1 protein. E2-induced expression of MKP1 and cotreatment with ICI 182.780 for 1 h inhibited E2-induced expression of MKP1.

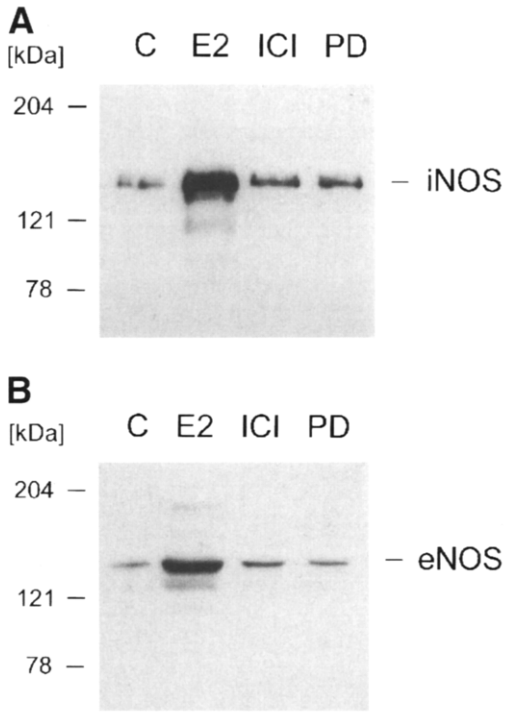

Fig. 6. Influence of ERK1/2 on E2-stimulated NO synthase expression. Adult female cardiomyocytes were incubated with E2 (10^{-9} M) for 1 h. Cell lysates were analyzed by Western blot analysis using specific antibodies against eNOS (A) or iNOS (B). Cotreatment with the pure antiestrogen ICI 182.780 (ICI, 10^{-8} M, 1 h) and with the MAPK pathway inhibitor PD 98059 (PD, 10^{-5} M, 1 h) inhibited upregulation of eNOS and iNOS expression.

estrogen on the myocardium such as the modulation of the L-type calcium current in cardiac myocytes obtained from human, rat or guinea pig heart have been described [12,13]. These genomic and non-genomic effects of estrogen may well play an important role in the cellular control of cardiac hypertrophy. The exact mechanisms of non-genomic effects of estrogen on the myocardium, however, remain to be clarified.

Here we show for the first time that E2 differentially activates intracellular signaling pathways which are involved in regulatory processes leading to cardiac hypertrophy [30]. Treatment of cardiac myocytes with E2 results in a rapid time-dependent activation of ERK1/2 and JNK. ERK1/2 and JNK activation occurred within 5–15 min, demonstrating that estrogen regulates non-genomic effects in cardiac myocytes. Interestingly, Migliaccio et al. [31] showed that the activation of the tyrosine-kinase/$p21^{ras}$/MAPK pathway in MCF-7 cells (mammary gland tumor cells) by estrogen requires the ligand occupancy of the estrogen receptor. We were able to demonstrate that the activity of ERK1/2 and JNK is completely inhibited by an ER-specific antagonist, indicating that the effect of E2 on ERK1/2 and JNK activity in cardiac myocytes is also mediated by the ER. Our findings raise a very intriguing question: how can the ER, which is usually known as a transcription factor, stimulate MAPK? A possible explanation is that the estrogen-ER complex may interact directly with signaling proteins. A second possible mechanism could involve the interaction between the ligand-activated ER and other peptide growth factors as shown recently by Karas et al. [32]. It is noteworthy that 17β-estradiol, while activating ERK and JNK, does not significantly stimulate the p38 pathway. p38 has been suggested to play a major role in the regulation of myocardial cell hypertrophy [30,33]. While activation of JNK and ERK generated only modest increases in cell size and natriuretic and α-skeletal actin promoter activity, induction of p38 significantly augmented cell size and promoter activity [34]. The identification of novel regulatory kinases (MEKKs) upstream of the signaling cascades mentioned above may help to elucidate these findings [35]. Each of these MEKKs is associated with a distinct MAPK cascade. There is at least one MEKK, MEKK3, which contains the ability to simultaneously activate ERK1/2 and JNK, but not p38 [36]. This serves as a likely explanation why both pathways are targets of estrogen stimulation. As we demonstrate that E2 significantly stimulates ERK1/2 and JNK but not p38, it is tempting to speculate whether estrogen only exerts a modest effect on mediators of cardiac myocyte hypertrophy such as the p38 pathway.

Duration of MAPK activation by extracellular stimuli is critical for cell signaling outcomes [37]. Inactivation of MAPK is mediated, in part, by dephosphorylation of MAPK by dual-specificity phosphatases (MKPs) that dephosphorylate both threonine and tyrosine residues phosphorylated by MEK [15]. Since MKP is principally regulated at the transcription level, we have studied cardiac MKP1 gene expression in response to estrogen. Estrogen-induced expression of MKP1 protein in cardiomyocytes correlates with the kinetics of dephosphorylation of MAPK. Furthermore, MKP1 expression was inhibited by ICI 182.780. Therefore, control of MAPK activity by E2 is exercised not only at the level of protein phosphorylation, but also through phosphatases such as MKP1 at the level of dephosphorylation. Taken together, these data demonstrate that 17β-estradiol influences both phosphorylation and deactivation of these protein kinases and that this process is also mediated through the ER.

Finally, we have studied the effect of MAPK signaling on the regulation of estrogenic target genes. Recently, we have shown that E2 stimulates expression of eNOS and iNOS in rat myocardium in vitro and in vivo [11]. Here we show that upregulation of iNOS and eNOS by 17β-estradiol in cardiomyocytes is inhibited in the presence of an inhibitor of the MAPK pathway (PD 98059). Therefore, a prerequisite for the elevated expression of both eNOS and iNOS seems to be the activation of ERK1/2 by estrogen.

In summary, we were able to demonstrate that estrogen activates ERK1/2 and JNK but not p38 in cardiac myocytes. Furthermore, 17β-estradiol induces MKP1 in cardiac myocytes and MEK blocker inhibits estrogen-induced NO synthase expression. These observations may help to dissect the role of estrogen in the pathogenesis of gender-based differences found in cardiac hypertrophy.

Acknowledgements: This work was supported by the Deutsche Forschungsgemeinschaft (Gr 729/8-1).

References

[1] Marcus, R., Krause, L., Weder, A.B., Dominguez-Mejia, A.N., Schork, D. and Julius, S. (1994) Circulation 90, 928–936.
[2] Gardin, J.M., Wagenknecht, L.E., Anton-Culver, H., Flack, J., Gidding, S., Jurosaki, T., Wong, N.D. and Manolio, T.A. (1995) Circulation 92, 380–387.
[3] Mosterd, A., D'Agostino, R.B., Silbershatz, H., Sytkowski, P.A.,

Kannel, W.B., Grobbee, D.E. and Levy, D. (1999) New Engl. J. Med. 340, 1221–1227.
[4] Kannel, W.B., Hjortland, M.C., McNamara, P.M. and Gordon, T. (1976) Ann. Intern. Med. 85, 447–452.
[5] Evans, R.M. (1988) Science 240, 889–895.
[6] Beato, M. (1989) Cell 56, 335–344.
[7] Walter, P., Green, S., Green, G., Krust, A., Bornert, J.-M., Jeltsch, J.M., Staub, A., Jensen, E., Scrace, G., Waterfield, M. and Chambon, P. (1985) Proc. Natl. Acad. Sci. USA 82, 7889–7893.
[8] Kuiper, G.G.J.M., Enmark, E., Pelto-Huikko, M., Nilsson, S. and Gustafsson, J.A. (1996) Proc. Natl. Acad. Sci. USA 93, 5925–5930.
[9] Kuiper, G.G.J.M., Carlsson, B., Grandien, K., Enmark, E., Häggblad, J., Nilsson, S. and Gustafsson, J.A. (1997) Endocrinology 188, 863–870.
[10] Grohé, C., Kahlert, S., Löbbert, K., van Eickels, M., Stimpel, M., Vetter, H. and Neyses, L. (1997) FEBS Lett. 416, 107–112.
[11] Nuedling, S., Kahlert, S., Loebbert, K., Doevendans, P.A., Meyer, R., Vetter, H. and Grohé, C. (1999) Cardiovasc. Res. (in press).
[12] Jiang, C., Poole-Wilson, P.A., Sarrel, P.M., Mochizuki, S., Collins, P. and MacLeod, K.T. (1992) Br. J. Pharmacol. 106, 739–745.
[13] Meyer, R., Linz, K.W., Surges, R., Meinardus, S., Vees, J., Hoffmann, A., Windholz, O. and Grohé, C. (1998) Exp. Physiol. 83, 305–321.
[14] Cobb, M.H. and Goldsmith, E.J. (1995) J. Biol. Chem. 270, 14843–14846.
[15] Derijard, B., Hibi, M., Wu, I., Barrett, T., Su, B., Deng, T., Karin, M. and Davis, R.J. (1994) Cell 76, 1025–1037.
[16] Kyriakis, J.M., Banerjee, P., Nikolakaki, E., Dai, T., Rubie, E.A., Ahmad, M.F., Avruch, J. and Woodgett, J.R. (1994) Nature 369, 156–160.
[17] Raingeaud, J., Gupta, S., Rogers, J.S., Dickens, M., Han, J., Ulevitch, R.J. and David, R.J. (1995) J. Biol. Chem. 270, 7420–7426.
[18] Cano, E. and Mahadevan, L.C. (1995) Trends Biochem. Sci. 20, 117–122.
[19] Sun, H., Charles, C.H., Lau, L.F. and Tonks, N.K. (1993) Cell 75, 487–493.
[20] Sun, H. and Tonks, N.K. (1994) Trends Biochem. Sci. 19, 480–485.
[21] Balligand, J-L. and Cannon, P.J. (1997) Arterioscler. Thromb. Vasc. Biol. 17, 1846–1858.
[22] Kelly, R.A., Balligand, J.-L. and Smith, T.W. (1996) Circ. Res. 79, 363–380.
[23] Claycomb, W.C. and Palazzo, M.C. (1980) Dev. Biol. 80, 466–482.
[24] Powell, T. (1985) Basic Res. Cardiol. 80, 8–12.
[25] Wakeling, A.E. and Bowler, J. (1992) J. Steroid Biochem. Mol. Biol. 43, 173–177.
[26] Dudley, D.T., Pang, L., Decker, S.T., Bridges, A.J. and Saltiel, A.R. (1995) Proc. Natl. Acad. Sci. USA 92, 7686–7689.
[27] Cuenda, A., Rouse, J., Doza, Y.N., Meier, R., Cohen, P., Gallagher, T.F., Young, P.R. and Lee, J.C. (1995) FEBS Lett. 364, 229–233.
[28] Kim-Schulze, S., Lowe, W.L. and Schnaper, W. (1998) Circulation 98, 413–421.
[29] Dunn, F.G. and Pfeffer, M.A. (1999) New Engl. J. Med. 16, 1279–1280.
[30] Sudgen, P.H. and Clerk, A. (1998) Circ. Res. 83, 345–352.
[31] Migliaccio, A., Di Domenico, M., Castoria, G., de Falco, A., Bontempo, P., Nola, E. and Auricchio, F. (1996) EMBO J. 15, 1292–1300.
[32] Karas, R.H., Gauer, E.A., Bieber, H.E., Baur, W.E. and Mendelsohn, M.E. (1998) J. Clin. Invest. 101, 2851–2861.
[33] Clerk, A., Michael, A. and Sudgen, P.H. (1998) J. Cell Biol. 142, 523–535.
[34] Zechner, D., Thuerauf, D.J., Hanford, D.S., McDonough, P.M. and Glembotski, C.C. (1997) J. Cell Biol. 139, 115–127.
[35] Minden, A., Lin, A., McMahon, M., Lange-Carter, C., Derijard, B., Davis, R.J., Johnson, G.L. and Karin, M. (1994) Science 266, 1719–1720.
[36] Ellinger-Ziegelbauer, H., Brown, K., Kelly, K. and Siebenlist, U. (1997) J. Biol. Chem. 272, 2668–2674.
[37] Marshall, C.J. (1995) Cell 80, 179–185.