17β-Estradiol Modulates Mechanical Strain-induced MAPK Activation in Mesangial Cells*

Received for publication, July 16, 2001, and in revised form, December 31, 2001
Published, JBC Papers in Press, January 2, 2002, DOI 10.1074/jbc.M106670200

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Gender is an important determinant of clinical outcome across a broad spectrum of kidney diseases, but the mechanism(s) responsible for the protective effect of female gender have not been fully elucidated. Remnant kidney glomerular injury is limited in female rats compared with male rats despite similar elevations in glomerular capillary pressure. In vitro, mechanical strain leads to the activation of p44/42 mitogen-activated kinase (p44/42 MAPK) and Jun N-terminal kinase/stress-activated protein kinase (SAPK) in glomerular mesangial cells (MC). Accordingly, we studied the effect of 17β-estradiol on mechanical strain-induced signal transduction in MC. Exposure of MC to mechanical strain increased p44/42 MAPK activation (3-fold) and SAPK activation (2.5-fold), and kinase activation was inhibited by pretreatment with 17β-estradiol (10⁻⁸ to 10⁻¹¹ M) for 24 h in a dose-dependent manner. Mechanical strain-induced nuclear translocation of p44/42 MAPK and SAPK and nuclear protein binding to AP-1 were also attenuated by 17β-estradiol. The inhibitory effects of 17β-estradiol were not reproduced by the cell-impermeable estrogen, BSA/17β-estradiol, nor did pretreatment with 17β-estradiol lead to actin cytoskeleton disassembly or impaired stress fiber formation. However, 17β-estradiol did increase base-line levels of the dual specificity phosphatase MKP-1. The inhibitory effects of 17β-estradiol on p44/42 MAPK activation and SAPK activation, translocation, and AP-1 binding were all abrogated by the estrogen receptor antagonist, ICI-182,780. We conclude that attenuation of mechanical strain-induced MAPK activation by 17β-estradiol is dependent on intracellular estrogen receptor. The attenuation of stretch-induced kinase activation may be due, at least in part, to an effect of 17β-estradiol on MKP-1 expression. Together, these findings add insight into the protective effect of gender on renal disease progression.

Chronic renal disease is relentlessly progressive after loss of a critical fraction of nephrons, and intraglomerular hemodynamics play a central role in the decline to end stage renal disease in this setting (1). This is most directly apparent in the ability of agents that prevent rises in intraglomerular pressure, such as angiotensin antagonists, to delay disease progression (2). More recently, other factors that impact progression of chronic renal disease have also been identified. Interestingly, it has been reported that females progress more slowly than males, and a protective effect of gender has been hypothesized (3). In support of this, estrogen has been found to limit both mesangial cell (MC) proliferation and matrix protein production (4). It was reported that acute estrogen exposure activated p44/42 (extracellular signal-regulated kinase 1/2) mitogen-activated protein kinase (MAPK) in resting MC and that this resulted in suppression of matrix protein synthesis (5). However, these studies also demonstrated that preincubation with estrogen antagonized p44/42 MAPK activation in response to angiotensin II or platelet-derived growth factor (5). Since estrogen exposure in vivo is not an acute event, it is probable that this latter observation is the more relevant.

MC are positioned as architectural supports for capillary loops and are therefore exposed in vivo to pulsatile stretch/relaxation (6). The effects of mechanical forces on MC in vitro can be modeled by culturing cells in wells with deformable bottoms, with application of a vacuum to the well to generate alternating cycles of strain and relaxation. Induction of mRNA for c-fos, the proto-oncogene and AP-1 transcription factor component, maximally at 30 min was the paradigmatic response observed with this system (6). MC proliferation (7) and collagenous and noncollagenous extracellular matrix protein synthesis, the sine qua non of sclerotic injury, are observed after 48 h of pulsatile stretch-relaxation (8).

We and others have studied the link between mechanical stress and c-fos induction in stressed MC (9, 10). We demonstrated increases in all three canonical MAPK pathways in response to strain (10) and demonstrated that MC proliferation in this setting was associated with MAPK activity (10, 11). We further showed that the ability of stretch to activate p44/42 MAPK was dependent on the actin cytoskeleton (12). Both p44/42 MAPK and SAPK are well recognized to lie upstream of AP-1 (13). In support of the importance of p44/42 MAPK/AP-1 signaling, glomerular p44/42 MAPK activation and AP-1 nuclear protein binding were shown in response to angiotensin II infusion (14). AP-1 activation may be important in the pathogenesis of glomerular sclerosis, since it has been shown to mediate transforming growth factor-β1 induction (15).

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¶ Supported by research funding from the Kidney Foundation of Canada.

** Supported by research funding from the Canadian Institutes of Health Research.

1 The abbreviations used are: MAPK, mitogen-activated protein kinase; MC, mesangial cell(s); kPa, kilopascals; BSA, bovine serum albumin; SAPK, stress-activated protein kinase; DTT, dithiothreitol; ER, estrogen receptor; PBS, phosphate-buffered saline; RT, reverse transcriptase.
Estradiol Attenuates Strain-induced Signaling in MC

Given the apparent protective effect of female gender on renal disease progression, the ability of preincubation with estrogen to down-regulate growth factor-induced p44/42 MAPK activity, and the induction of MAPK by MC stress, we postulated that estrogen was likely to inhibit MC MAPK activation in stretched cells. Accordingly, we studied the effect of estrogen on stretch-induced activation of p44/42 MAPK and SAPK in MC.

**MATERIALS AND METHODS**

**Cell Culture**

Sprague-Dawley rat MC were cultured in Dulbecco’s modified Eagle’s medium supplemented with 20% fetal calf serum (Invitrogen), streptomycin (100 μg/ml), penicillin (100 units/ml), and 2 mM glutamine at 37 °C in 5% air, 5% CO₂. Experiments were carried out in cells between passages 15 and 20.

**Application of Strain/Relaxation**

MC (5 × 10⁵/well) were plated on six-well plates with flexible bottoms coated with bovine type I collagen (Flexcell International Corp., McKeesport, PA). Cells were grown to confluence for 72 h and then rendered quiescent by incubation for 24 h in Dulbecco’s modified Eagle’s medium with 0.5% fetal calf serum. To characterize the time of maximum response, cells were initially exposed to cycles of strain/relaxation for periods of 2, 5, 10, 30, and 60 min, generated by a cyclic vacuum produced by a computer-driven system (Flexcell Strain Unit 2000; Flexcell). To establish if more than one peak of MAPK activation exists, a longer time course with stretch exposure for 5, 10, 30, and 60 min and 4 and 24 h was conducted. For all experiments, plates were exposed to continuous cycles of strain/relaxation, each cycle consisting of 0.5 s of strain and 0.5 s of relaxation, for a total of 60 cycles/min. Initially, vacuum pressures used were −10 to −27 kPa, inducing a 16–28% elongation in the diameter of the surface. Subsequent experiments were performed at the time and strain level of maximal response, 10 min and −27 kPa (average 28% elongation in diameter of the plate).

To study the effects of estrogen on stretch-activated MAPK, MC were exposed to 17β-estradiol or to the cell-impermeable 17β-estradiol/bovine serum albumin (BSA) (both from Sigma) were added at the indicated concentrations 24 h prior to the initiation of stretch protocols. ICI-182,780 (Tocris Cookson, St. Louis, MO), a high affinity estrogen receptor antagonist, was added 24 h prior to the initiation of stretch protocols where indicated.

**MAPK Phosphorylation and Activities**

**Protein Isolation and Western Blotting**—Initially, the time course and concentration dependence of MAPK activities in response to stretch were studied, and subsequent experiments were performed at −27 kPa at 10 min. Cultures were serum-starved overnight prior to stretch protocols. After stretch protocols with or without estrogen and inhibitors, medium was removed, and the cells were washed once with ice cold PBS. Cells were resuspended in a buffer containing 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 1 mM EGTA, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 2 mM DTT, 1 mM Na₃VO₄, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, and 20 μg/ml aprotinin. Cell lysate (50 μg/lane) was separated by SDS-PAGE and transferred onto Immobilon-P membranes (Millipore Corp.). After blocking with 5% skim milk, primary antibodies were applied. The following antibodies were used for immunoblots: anti-p42/p44 extracellular signal-regulated kinase (1:500), anti-SAPK (1:500), anti-phospho-Thr183/Tyr185 monoclonal antibody (1:200) (New England Biolabs) with 25 μM 3-γ-sinar sample buffer (187.5 mM Tris-HCl (pH 6.8), 6% (w/v) SDS, 30% glycerol, 150 mM DTT, 0.3% (w/v) bromphenol blue), boiled for 5 min, vortexed, and then microcentrifuged for 2 min. 20 μl of sample was then run on a SDS-PAGE gel. Membranes were then probed with nitrocellulose, incubated overnight at 4 °C with phosphospecific anti-Elk (Ser383) antibody (1:1000) and visualized as above. To assay SAPK activity, a “pull-down” SAPK assay was employed. After protein isolation from total cell lysate with 2 μg of c-Jun fusion protein beads (New England Biolabs) were added to 250 μg of cell lysate protein and incubated overnight at 4 °C. Lysate was then centrifuged for 30 s to recover the beads and washed twice with 1% lysia buffer. The pellet was then resuspended in kinase buffer and boiled as previously. 20 μl of sample was run on a 12% SDS-PAGE gel. Blotting and detection were performed as above, except that the primary antibody was phosphospecific c-Jun (Ser383) at 1:1000 dilution.

**Fluorescence Microscopy**

**MAPK Nuclear Translocation**—After each strain protocol with or without estrogen and inhibitors, cells were washed three times with PBS and fixed with 3.7% formaldehyde (30 μl/well) for 10 min at room temperature. Cells were washed three times with PBS and then permeabilized in 100% methanol for 5 min at −20 °C, washed again with PBS, and incubated with anti-phospho-p44/42 MAPK (1:200) with monoclonal mouse anti-ER (1:500; Affinity BioReagents) as the primary antibody.

**Radiolabeled Oligonucleotides**

32P-labeled AP-1 consensus oligonucleotides were prepared by incubating 2 μl of consensus oligonucleotide (1.75 pmol/μl; Promega), 1 μl of T4 polynucleotide kinase 10× buffer, 1 μl of [γ-32P]ATP (3,000 Ci/ml) (Amersham Biosciences), and 5 μl of nuclease-free water for 10 min at 37 °C. The reaction was stopped by adding 1 μl of 0.5 M EDTA. Unlabeled [32P]ATP was removed from the oligonucleotide mixture with Chroma-Spin STE-10 columns (CLONTECH, Palo Alto, CA).

**Nuclear Protein Binding to AP-1 Consensus Sequences**—After each strain protocol, MC were washed in cold PBS, and nuclear extracts were prepared by lysis in hypotonic buffer (20 mM Hepes, pH 7.9, 1 mM EDTA, 1 mM EGTA, 20 mM Na₃VO₄, 1 mM NaF, 1 μM Na₄P₂O₇, 1 mM Na₃VO₄, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin A, 0.6% Nonidet P-40), homogenized, and sedimented at 16,000 × g for 20 min at 4 °C. Pelleted nuclei were resuspended in hypotonic buffer containing 0.42 mM NaCl, 20% glycerol and rotated for 30 min at 4 °C. After centrifugation for 20 min at 16,000 × g, the supernatant containing nuclear proteins was collected, and protein concentration was measured with the Bio-Rad assay kit. Radiolabeled AP-1 consensus oligonucleotides were preincubated with 2 μl of consensus oligonucleotide (1.75 pmol/μl; Promega), 1 μl of T4 polynucleotide kinase 10× buffer, 1 μl of [γ-32P]ATP (3,000 Ci/ml) (Amersham Biosciences), and 5 μl of nuclease-free water for 10 min at 37 °C. The reaction was stopped by adding 1 μl of 0.5 M EDTA. Unlabeled [32P]ATP was removed from the oligonucleotide mixture with Chroma-Spin STE-10 columns (CLONTECH, Palo Alto, CA).

Nuclear proteins (3 μg) were incubated with 2 μg of poly(dI- dC)·poly(dI·dC) (Amersham Biosciences) in binding buffer (20 mM HEPES, pH 7.9, 1.8 mM MgCl₂, 2 mM DTT, 0.5 EDTA, 0.5 μg/ml BSA) for 30 min at room temperature and then reacted with radiolabeled consensus oligonucleotides at room temperature for 20 min (50,000–100,000 cpm). Reaction mixtures were electrophoresed in a 6% polyacrylamide gel and autoradiographed. Competition experiments were performed with a 100-fold excess of unlabeled AP-1 consensus oligonucleotides.

**RNA Isolation and Semiquantitative Reverse Transcriptase (RT)-PCR**

Total RNA from MC was isolated by the single step method of Chomczynski and Sacchi (16) as we have described (17). Isolated RNA was stored in diethyl pyrocarbonate-treated water at −80 °C. The pu-
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Statistical Analysis

Statistical analyses were performed with the INSTAT statistical package (GraphPad Software Inc., San Diego, CA). The difference between means was analyzed using the Bonferroni Multiple comparison test. Significance was defined as p < 0.05.

RESULTS

Estrogen Dose-dependently Attenuates Strain-induced MAPK Activity in MC—As in previous studies, we observed time- and magnitude-dependent strain activation of p44/42 MAPK and SAPK (10, 12), maximally at 5–20 min and at ~27 kPa pressure (data not shown). Only one peak of activation for both kinases was observed over a 24-h period of strain at ~27 kPa (Fig. 1). Preincubation for 24 h with 17β-estradiol dose-dependently inhibited the activity of both MAPK cascades at 10 min of ~27 kPa stretch (Fig. 2, A and B), maximally at 10−8 M. Consequently, subsequent studies used this concentration of 17β-estradiol and stretch was applied for 10 min at ~27 kPa and 60 Hz.

Cell Entry and Time Are Required for the Effect of Estrogen—It has become apparent in recent years that both cell surface and cytoplasmic estrogen receptors exist, and some of the cardioprotective effects of estrogen have been ascribed to the former. In the most well studied example, 17β-estradiol induced endothelial nitric-oxide synthase through rapid (5 min) MAPK activation (18, 19). Similar rapid actions of estrogen have been observed in neuronal and breast cancer cells (20, 21). In breast cancer cells, activation of MAPK was tyrosine kinase- and Ras-dependent, suggesting activation of a pathway similar to that employed by classic growth factors (21). The existence of membrane estrogen receptors has been known for 20 years (22), but only recently has their potential importance been appreciated. Both the classic estrogen receptor (ER-α) and a second isotype, ER-β, are expressed in MC (23).

Accordingly, we wished to determine whether the ability of 17β-estradiol to inhibit MAPK activity in MC was receptor-mediated and whether cell surface or cytoplasmic receptors were most important. To address this question, we employed a specific estrogen receptor antagonists, ICI 182,780, and a cell-impermeable estrogen agonist, 17β-estradiol/BSA. Co-incubation with ICI 182,780 (5 × 10−7 M) prevented the inhibitory actions of 17β-estradiol on p44/42 MAPK and SAPK activity in stretch cells (Fig. 3, A and B), indicating that 17β-estradiol acted through estrogen receptors. Expression of estrogen receptor α was not affected by 17β-estradiol (Fig. 4). 17β-Estradiol/BSA had significantly less inhibitory effect when compared with 17β-estradiol (Fig. 5, A and B), indicating that cell penetration was required for full inhibitory actions of 17β-estradiol. Some ability to attenuate stretch-induced p44/42 MAPK and SAPK activation was observed with 17β-estradiol/BSA, so we cannot exclude the possibility of a membrane receptor-mediated component in this circumstance. The importance of non-nuclear activation was emphasized, however, by our observations that 2 h of preincubation with 17β-estradiol was unable to inhibit p44/42 MAPK (Fig. 6) or SAPK (data not shown) signaling in response to MC stretch.

Estrogen Prevents Strain-induced MAPK Nuclear Localization—We have observed prompt nuclear localization of phosphorylated p44/42 MAPK and phosphorylated SAPK in response to stretch (12, 24). Consequently, we sought to determine whether 17β-estradiol would prevent nuclear localization of phospho-MAPKs and whether this was a receptor-mediated event. Figs. 7 and 8 demonstrate that stretch-induced p44/42 MAPK and SAPK activation and nuclear localization are prevented by preincubation with 10−8 M 17β-estradiol. The addition of the specific estrogen receptor antagonist ICI 182,780 (5 × 10−7 M) restored activation and nuclear localization of phospho-MAPKs, indicating that the inhibitory effect of estrogen resides in receptor-mediated effects.
AP-1 nuclear protein binding in stretched MC (12). Accordingly, we sought to determine the effect of 17β-estradiol on AP-1 nuclear protein binding in MC exposed to cyclic strain. Preincubation with 10^{-8} M 17β-estradiol for 24 h prior to stretch substantially decreased AP-1 nuclear protein binding after 10 min of stretch (Fig. 9). Again, co-incubation with ICI 182,780 restored AP-1 binding, indicating the importance of estrogen receptors in this effect.

Estrogen Attenuates the Induction of c-fos in Response to Strain—Both p44/42 MAPK and SAPK signal to induce Fos and Jun protein expression and thus AP-1 transactivational activity (13). Indeed, increases in c-fos expression represented the first important MC response to mechanical strain described (6). Consequently, we sought to determine whether 17β-estradiol could inhibit c-fos expression in stretched MC. Indeed, preincubation for 24 h with 10^{-8} M 17β-estradiol abrogated the increase in c-fos expression observed with RT-PCR after 2 h of cyclic strain (Fig. 10). ICI 182,780 blocked the inhibitory effect of 17β-estradiol, indicating once again the importance of estrogen receptors in mediating this effect.

Estrogen Does Not Disrupt Stretch-induced Cytoskeletal Arrangement—In recent work, we demonstrated that stretch-induced p44/42 MAPK activation in MC was absolutely dependent on the presence of an intact actin cytoskeleton (12). Consequently, we sought to determine whether the ability of 17β-estradiol to disrupt such activation rested in effects on the actin cytoskeleton. Phalloidin staining revealed prompt (10
specific activity for phospho-p44/42 MAPK (26) was unaffected. Protein expression of MKP-3, which has resulted in an almost 2-fold increase in MKP-1 protein levels as incubation with 17\textsuperscript{estradiol} (10\textsuperscript{-8} and 10\textsuperscript{-7} m) for 24 h resulted in an almost 2-fold increase in MKP-1 protein levels as seen in Fig. 12A. Protein expression of MKP-3, which has specific activity for phospho-p44/42 MAPK (26) was unaffected (Fig. 12B).

**DISCUSSION**

In the best characterized animal model of chronic renal failure, the subtotally nephrectomized rat, increased glomerular capillary pressure (as little as 20%) triggers MC responses that ultimately result in glomerulosclerosis (8, 27). However, it has been observed that female rats subjected to subtotal nephrectomy suffer only a fraction of the sclerotic injury observed in male rats (28). In support of this, differences in glomerular hemodynamics have also been observed in female rats, with a tendency to vasorelaxation (29).

The MC is central to the injurious glomerular response to insults that result in progressive renal failure, such as subtotal nephrectomy. In vitro studies of the application of cyclic mechanical strain to MC have demonstrated that this stimulus results in MC proliferation (30) and production of collagenous protein (7), thus mimicking the in vivo responses observed after increases in intraglomerular pressure. We and others have repeatedly observed activation of MAPKs in response to either cyclic or static MC strain and have shown such activation to signal proliferation and matrix production (9, 11, 31, 32). Given the apparent protection afforded by female gender to 5/6 nephrectomy, we hypothesized that estrogen would inhibit MAPK activation in stretched MC and thus prevent proliferation.

Indeed, the first major finding of the current study was that 24-h preincubation with 17\textsuperscript{estradiol} prevented the usual prompt activation of p44/42 MAPK and SAPK observed after the initiation of strain. Similarly, the nuclear translocation of phosphorylated kinases was also prevented. Tellingly, preincubation with 17\textsuperscript{estradiol} for 2 h was unable to affect strain activation, suggesting that the observed effect of estrogen was not an acute event but probably involved genomic actions. This was supported by two other observations, that inhibition of 17\textsuperscript{estradiol} binding to receptors by ICI 182,780 restored MAPK activation in stretched cells and that a cell-impermeable estrogen agonist, 17\textsuperscript{estradiol}/BESA, had significantly less inhibitory effect on strain-induced MAPK signaling. These data indicate that 17\textsuperscript{estradiol} enters the cell and binds to cytoplasmic or nuclear receptors to exert inhibitory actions on MAPK signaling.

A number of studies have examined the effect of estrogen on vascular smooth muscle cells and MC, and estrogen has been shown to inhibit serum and growth factor-induced p44/42 MAPK activity. In addition, 17\textsuperscript{estradiol} limits tumor necrosis...
factor α-induced SAPK activation in chondrocytes. Interestingly, estrogen was found to inhibit type I collagen synthesis in quiescent MC in response to transforming growth factor-β1, an effect that is dependent on Sp1 (5). Our studies of the effects of 17β-estradiol on strain-induced kinase activation in MC are in accord with these observations. Short term studies have, however, also revealed that 17β-estradiol can activate MAPKs (5). This effect was also observed with selective estrogen receptor modulators (23). Although two or more peaks of MAPK activity may occur in MC in response to some stimuli (33), we observed only one peak of MAPK activation over 24 h of strain.

The two well established nuclear estrogen receptors, ERα and ERβ, are known to exist in murine MC (23). The actions of ERα after ligation by estrogenic agonists such as 17β-estradiol are well defined. One target of the activated nuclear ERα is AP-1. An interaction between the ERα-estrogen complex can modify binding of the Fos-Jun heterodimer to AP-1 consensus sequences (34). We have demonstrated p44/42 MAPK-dependent induction of AP-1 nuclear protein binding in stretched MC (12). Consequently, we reasoned that 17β-estradiol might affect AP-1 nuclear protein binding to consensus sequences in stretched MC. Consistent with its inhibitory effects on p44/42 MAPK and SAPK activation, 17β-estradiol also prevented AP-1 nuclear protein binding in stretched MC. As expected, this effect depends on an interaction of 17β-estradiol with the ER,

**FIG. 6.** Stretch-induced p44/42 MAPK phosphorylation in MC is not prevented by short term incubation with 17β-estradiol. Serum-starved MC were incubated with 17β-estradiol (10⁻⁸ M) for 2 h and then exposed to a 27-kPa stretch at 60 Hz for 10 min. p44/42 MAPK phosphorylation was measured by Western blot with an antibody specifically recognizing phosphorylated extracellular signal-regulated kinase (Thr202/Tyr204).

**FIG. 7.** ICI-182,780 restores stretch-induced phospho-p44/42 MAPK nuclear translocation in MC treated with 17β-estradiol. MC were incubated with anti-phospho-p44/42 MAPK, washed, incubated with a goat anti-rabbit IgG conjugate, and then visualized using confocal microscopy. Unstretched cells (A) show only light nuclear staining. Application of 27-kPa stretch at 60 Hz for 10 min led to prompt induction and nuclear translocation of phospho-p44/42 MAPK (B). This was completely prevented by preincubation with 17β-estradiol (10⁻⁸ M, 24 h) (C). Co-incubation with ICI-182,780 (5 × 10⁻⁷ M, 24 h) restored the stretch-induced nuclear translocation (D).

**FIG. 8.** ICI-182,780 restores stretch-induced phospho-SAPK nuclear translocation in MC treated with 17β-estradiol. MC were incubated with anti-phospho-SAPK, washed, incubated with a goat anti-rabbit IgG conjugate, and then visualized using confocal microscopy. Unstretched cells (A) show only light nuclear staining. Application of 27-kPa stretch at 60 Hz for 10 min led to prompt induction and nuclear translocation of phospho-SAPK (B). This was completely prevented by preincubation with 17β-estradiol (10⁻⁸ M, 24 h) (C). Co-incubation with ICI-182,780 (5 × 10⁻⁷ M, 24 h) restored the stretch-induced nuclear translocation (D).
since it could be prevented by ICI 182,780. Concordantly, c-fos induction in this setting was also inhibited by 17β-estradiol.

We and others have shown that MAPK-AP-1 signaling is a critical inducer of proliferation in stretched MC (9, 12, 35). The ability of 17β-estradiol to inhibit this signaling pathway was not via up-regulation of ERα, since incubation of MC with 10^{-8} M 17β-estradiol for 24 h did not affect ERα expression by Western blot.

We have demonstrated that stretch-induced MAPK activation depends on the presence of an intact actin cytoskeleton (12). It has been observed that estrogen leads to actin depolymerization in cervical epithelia (36) and that cytoplasmic estrogen receptors may interact with RhoGTPases (37). Consequently, we sought to determine whether the ability of 17β-estradiol to prevent stretch-induced MAPK signaling in MC was secondary to effects on the actin cytoskeleton. Phalloidin staining revealed that actin stress fibers formed normally (within 10 min) in stretched MC in the presence of estrogen, indicating that other mechanisms must account for estrogen’s inhibition of MAPK signaling in this setting.

Recently, Nuedling et al. (38) have shown that MKP-1 is up-regulated by 17β-estradiol in unstretched cardiac myocytes, an effect seen as early as 30 min after stimulation and associated with a concurrent decrease in estrogen-mediated p44/42 MAPK activation in these cells. MKP-1 is the prototype of the...
MAPK dual specificity phosphatase family and may inactivate both p44/42 MAPK and SAPK (25). Consequently, we investigated the effects of 17β-estradiol on MKP-1 expression in MC and found that 24-h incubation with 17β-estradiol induced protein levels of MKP-1. No such effect was observed with the phopho-p44/42 MAPK-specific phosphatase MKP-3. Although protein levels of MKP-1, No such effect was observed with the phopho-p44/42 MAPK-specific phosphatase MKP-3. Although additional mechanisms may exist whereby 17β-estradiol inhibits its stretch-induced signaling in MC, this effect on MKP-1 may be responsible for the attenuation of stretch-induced MAPK activation.

In summary, the data presented in this study suggest a mechanism by which estrogen may exert protective effects in progressive renal disease (i.e., through the inhibition of stretch-induced MAPK activation). The inhibition of stretch-induced MAPK activation by 17β-estradiol is dependent on intracellular ER receptors and is time-dependent. 17β-estradiol does not inhibit strain-induced stress fiber formation of the actin cytoskeleton but does increase base-line expression of MKP-1. Further studies will be necessary to define the mechanism(s) whereby estrogen up-regulates this dual specificity phosphatase.

REFERENCES

1. Brenner, B. M. (1985). Am. J. Physiol. 249, F324–F337
2. Anderson, S., Meyer, T. W., Rennke, H. G., and Brenner, B. M. (1985) J. Clin. Invest. 76, 612–619
3. Silbiger, S. R., and Neugarten, J. (1995) Am. J. Kidney Dis. 25, 515–533
4. Kwan, G., Neugarten, J., Sherman, M., Ding, Q., Fotadar, U., Lei, J., and Silbiger, S. (1996) Kidney Int. 50, 1173–1179
5. Neugarten, J., Medve, I., Lei, J., and Silbiger, S. R. (1999) Am. J. Physiol. 277, F575–F581
6. Akai, Y., Homma, T., Burns, K. D., Yasuda, T., Badr, K. F., and Harris, R. C. (1994) Am. J. Physiol. 267, C482–C490
7. Harris, R. C., Haralson, M. A., and Badr, K. F. (1992) Lab. Invest. 66, 548–554
8. Riser, B. L., Cortes, P., Zhao, X., Bernstein, J., Dumler, F., and Narines, R. G. (1992) J. Clin. Invest. 90, 1932–1934
9. Kawata, Y., Mizukami, Y., Fujii, Z., Sakamura, T., Yoshida, K., and Matsuoka, M. (1996) J. Biol. Chem. 273, 16905–16912
10. Ingram, A., Thai, K., Ly, H., Kang, M., and Scholey, J. W. (1999) Kidney Int. 55, 476–485
11. Ingram, A. J., James, L., Ly, H., Thai, K., Cai, L., and Scholey, J. W. (2000) Kidney Int. 58, 1067–1077
12. Ingram, A. J., James, L., Cai, L., Thai, K., Ly, H., and Scholey, J. W. (2000) J. Biol. Chem. 275, 40301–40306
13. Karin, M. (1995) J. Biol. Chem. 270, 16483–16486
14. Hamaguchi, A., Kim, S., Yano, M., Yamanaka, S., and Iwao, H. (1998) J. Am. Soc. Nephrol. 9, 372–380
15. Kim, S. J., Angel, P., Laffaz, R., Hattori, K., Kim, K. Y., Sporn, M. B., Karin, M., and Roberts, A. B. (1990) Mol. Cell. Biol. 10, 1492–1497
16. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
17. Ingram, A., Parbtani, A., Thai, K., Ly, H., Shankland, S. J., Morrissey, G., and Scholey, J. W. (1999) Kidney Int. 48, 1857–1865
18. Chen, Z., Yuhanna, I. S., Galcheva-Gargova, Z., Karas, R. H., Mendelsohn, M. E., and Shaul, P. W. (1989) J. Clin. Invest. 83, 401–406
19. Goetz, R. M., Thatte, H. S., Prabhakar, P., Cho, M. R., Michel, T., and Golan, D. E. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 2788–2793
20. Singer, C. A., Figueroa-Maset, X. A., Batcher, R. H., and Dorsa, D. M. (1999) J. Neurosci. 19, 2455–2463
21. Migliaccio, A., Di Domenico, M., Casteria, G., de Falco, A., Bonfemeto, P., Nola, E., and Auricchio, F. (1996) EMBO J. 15, 1292–1300
22. Pietras, R. J., and Szego, C. M. (1971) Nature 265, 69–72
23. Neugarten, J., Acharya, A., Lei, J., and Silbiger, S. (2000) Am. J. Physiol. 279, F309–F318
24. Ingram, A. J., James, L., Thai, K., Ly, H., Cai, L., and Scholey, J. W. (2000) Am. J. Physiol. 279, F243–F251
25. Haneda, M., Sugimoto, T., and Kikkkawa, R. (1999) Eur. J. Pharmacol. 365, 1–7
26. Muda, M., Theodosiou, A., Rodrigues, N., Boschert, U., Camps, M., Gillieron, C., Davies, K., Ashworth, A., and Arkinstall, S. (1996) J. Biol. Chem. 271, 27205–27208
27. Yasuda, T., Kondo, S., Homma, T., and Harris, R. C. (1996) J. Clin. Invest. 98, 1991–2000
28. Lomholt, J. R., Adler, S. G., Anderson, P. S., Nast, C. C., Olsen, D. R., and Glasscock, R. J. (1988) J. Lab. Clin. Med. 114, 66–74
29. Munger, K., and Baylis, C. (1988) Am. J. Physiol. 254, F233–F241
30. Harris, R. C., Akai, Y., Yasuda, T., and Homma, T. (1984) Kidney Int. Suppl. 45, 17–21
31. Ingram, A. J., James, L., Ly, H., Thai, K., and Scholey, J. W. (2000) Kidney Int. 58, 1431–1439
32. Ishida, T., Haneda, M., Maeda, S., Koya, D., and Kikkawa, R. (1999) Diabetes 48, 586–592
33. Bonachea, A., Romagnani, P., Romanielli, R. G., Efeou, E., Annunziato, F., Lasagui, L., Francalanci, M., Serio, M., Laffi, G., Pinzani, M., Gentilini, P., and Marra, F. (2001) J. Biol. Chem. 276, 9945–9954
34. Kamiyama, Y., Kawamura, H., Watada, H., Imao, R., Iwama, N., Morishima, T., Yamanaka, K., Kajimoto, Y., and Kamada, T. (1994) J. Biol. Chem. 269, 16433–16442
35. Yasuda, T., Akai, Y., Kondo, S., Becker, B. N., Homma, T., Owada, S., Ishida, M., and Harris, R. C. (1990) Contrib. Nephrol. 118, 222–228
36. Gorodeski, G. I. (2000) Am. J. Physiol. Cell Physiol. 279, C2028–C2036
37. Su, L., Knoblauch, R., and Garabedian, M. J. (2000) J. Biol. Chem. 276, 3231–3237
38. Nuedling, S., Kahle, S., Lobbert, K., Meyer, R., Vetter, H., and Grohe, C. (1999) FEBS Lett. 454, 271–276

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J. Biol. Chem. 2002, 277:9387-9394.
doi: 10.1074/jbc.M106670200 originally published online January 2, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M106670200

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