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Effects of 17β-estradiol and progesterone on mitogen-activated protein kinase expression and activity in rat uterine smooth muscle

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Abstract

Activation of mitogen-activated protein kinases (MAPKs) is a critical event in mitogenic signal transduction. MAPKs are activated by tyrosine phosphorylation and translocate to different cellular compartments affecting protein function and gene expression. MAPK expression and activity was examined in uterine smooth muscle from rats pretreated with estradiol-17β alone or with estradiol-17β and progesterone. MAPK expression was detected by immunoblotting using erk1/2 antibodies. MAPK activity was detected by measurement of the phosphorylation of a MAPK-specific peptide sequence of myelin basic protein. Steroid treatment caused a modest (20%) decline in erk 1 and 2 expression in membrane and cytosolic fractions. Both estrogen and progesterone increased MAPK tyrosine phosphorylation and membrane-associated MAPK activity. Steroid treatment increased cytosolic MAPK tyrosine phosphorylation, but not enzymatic activity. These data suggest that gonadal steroid hormones, which stimulate uterine hypertrophy, may exert their hypertrophic effects by increasing MAPK activity.

Keywords: Mitogen-activated protein kinase; Smooth muscle, uterine; Steroid; Estradiol; Progesterone

1. Introduction

Hypertrophy, an increase in cell size without cell division, is one of the primary cellular adaptations that uterine smooth muscle undergoes during pregnancy. The hypertrophic response is characterized by an increase in cellular protein synthesis, increased myocyte contractile protein content, increased organization of contractile proteins, and transitions in the transcription of structural, contractile protein genes and proto-oncogenes. From in vivo and in vitro studies, a smooth muscle hypertrophic response can be activated by mechanical (e.g. external load, stretch) (Owens, 1991; Dzau, 1993) and biochemical inputs (e.g. steroids, angiotensin II, basic fibroblast growth factor, serotonin) (Ashen and Hamlyn, 1994; Glassberg et al., 1994; Lee et al., 1994; Morishita et al., 1994).

Little is known regarding the molecular mechanisms which control uterine smooth muscle cell hypertrophy. Activation of the mitogen-activated protein kinases (MAPK), also known as extracellular regulated kinases (erks), in vascular smooth muscle and cardiac myocytes has been suggested to be involved in the initiation of the hypertrophic response (Langan et al., 1994; Morinelli et al., 1994).

The mitogen-activated protein kinase signaling cascade is a common pathway linking the cell surface with the nucleus (Blenis, 1993; Davis, 1993). Many growth factors, cytokines, and vasoactive substances exert mitogenic effects by activating mitogen-activated protein kinases. Of particular relevance to muscle hypertrophy is that mitogen-activated protein kinases are acutely activated by mechanical loading or by agonists which induce myocyte hypertrophy in vitro (Bogoyevitch et al., 1993; Yamazaki et al., 1993). Inhibition of mitogen-activated protein kinase activity prevents α-adrenergic-induced hypertrophy in cultured cardiac myocytes (Thorburn and Thorburn, 1994). Mitogen-activated protein kinases phosphorylate nuclear substrates which are transcription factors (c-jun, c-myc, c-fos) involved in the expression of genes characteristic of the hypertrophic response (Jamal and Ziff, 1990). Additionally, mitogen-activated protein kinases phosphorylate microtubule-associated proteins affecting cytoskeletal orientation and integrity (Davis, 1993). It is unclear whether

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mitogen-activated protein kinases mediate changes in cytoskeletal organization associated with cell hypertrophy.

This study was carried out to examine whether gonadal steroid-induced uterine hypertrophy is associated with an activation of the mitogen-activated protein kinase pathway. The effect of in vivo treatment with 17β-estradiol and progesterone on the expression, intracellular distribution and enzymatic activity of two mitogen-activated protein kinases, erk1 and erk2, was examined in the rat uterus.

2. Materials and methods

2.1. Tissue preparation

Virgin female Sprague Dawley rats weighing 175–200 g were purchased ovariectomized from Taconic (Germantown, NY, USA). Upon arrival, animals were housed under conditions of controlled temperature and lighting. Food and water was provided ad libitum. Ten days after shipment, animals were treated with estrogen or estrogen and progesterone. Animals referred to as 'estrogen-dominated' were injected once every day with estradiol-17β (200 μg/kg s.c.) for 4 days, while 'progesterone-dominated' animals received estradiol-17β and progesterone (200 μg/kg and 20 mg/kg, respectively) for an additional 4 days. Control animals did not receive any injections. Employing this protocol, mean uterine wet weight was over 2-fold greater from estrogen (0.321 ± 0.003 g; $n = 33$)- and progesterone (0.322 ± 0.003 g; $n = 34$)-dominated animals relative to control animals (0.126 ± 0.002 g; $n = 50$). All animal studies were reviewed and approved by the institutional committee on animal use and care.

Twenty-four hours following the last hormone injection, animals were killed by decapitation. Both uterine horns were rapidly excised (0.5 cm from both the ovarian ligation and the uterine bifurcation) and placed in a physiologic salt solution at 37°C of the following composition (mM): NaCl, 118; KCl, 4.7; $MgCl_2$, 1.1, $CaCl_2$, 1.8; $NaHCO_3$, 25; KH_2PO_4, 1.0; and dextrose, 5.6. The uterine horns were carefully cleaned of surrounding fat and connective tissues and opened longitudinally along the mesenteric border. The lumenal surface of the uterus was gently rubbed with a cotton swab to remove the endometrium.

2.2. Preparation of membrane and cytosolic fractions

Tissues collected from at least 10 animals per treatment group were pooled for preparation of subcellular fractions. Membrane fractions were prepared according to procedures previously described (Ruzycky and Triggle, 1987) with the following modification. Tissues were homogenized in a buffer containing 50 mM Tris pH 7.4, 1 mM EDTA, 0.5 μg/ml leupeptin, 0.7 μg/ml pepstatin, and 20 μg/ml phenylmethylsulfonyl fluoride. Membrane pellets were resuspended in 50 mM Tris pH 7.4 and stored at −80°C until use. The cytosolic supernatant was further concentrated using a Centriprep concentrator with a 3000 molecular weight cutoff (Amicon, Beverly, MA, USA) to a protein concentration of at least 10 mg/ml and stored at −80°C until use.

Protein concentration of subcellular fractions was determined on each aliquot at time of use by the bicinchoninic acid (BCA) assay (Pierce; Rockford, IL, USA) using bovine serum albumin as a standard reference.

2.3. Immunoblots

Fifteen micrograms of protein were applied to a 10% sodium dodecyl sulfate-polyacrylamide minigel (1 mm thickness) and resolved at 200 V for approximately 45 min (Laemmli, 1970). Proteins were transferred electrophoretically to nitrocellulose membranes. Transfer efficiency was monitored using prestained molecular weight standards (Bio-Rad; Hercules, CA, USA). After transfer, the membranes were blocked with 10% goat serum/0.1% Tween 20/buffered saline (PBS; 500 mM Tris, 2 M NaCl, pH 7.0) for 1 h at room temperature. The membranes were then incubated with protein-specific polyclonal antibodies (1 : 400 dilution in 1% bovine serum albumin, 2% goat serum, 0.05% Tween 20 PBS) for 3 h at room temperature. After three washes with 0.1% Tween 20, blots were incubated for 1 h with horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse antibodies where appropriate. Peroxidase activity was detected using Renaissance chemiluminescent reagents (NEN, Boston, MA, USA). Intensity of immunoreactive bands of interest was measured using a Harmony Group Image Analysis System (Videk Corp., Rochester, NY, USA).

2.4. Immunoprecipitation (IP)

Subcellular fractions (140 μg) were incubated in IP buffer (20 mM Tris pH 7.4, 300 mM NaCl, 2 mM EDTA, 2 mM EGTA, 0.4 mM sodium orthovanadate, 0.4 mM phenylmethylsulfonylfluoride, 0.5% NP-40, and 1% Triton X-100) for 30 min on ice. Samples were then incubated with 4 μl of PY20 antibody overnight at 4°C. Following a subsequent 1 h incubation with 10 μl of Protein A sephararose CL-4B, samples were centrifuged for 5 min, supernatants carefully removed, and pellets washed 3 times with IP buffer. Pelleted proteins were resuspended in 50 μl of SDS gel sample buffer (0.5 M Tris pH 6.8, 10% SDS, 0.5% bromophenol blue), boiled for 5 min, and electrophoresed as described.

2.5. Assay of MAPK enzymatic activity

Paired membrane and cytosolic fractions (35 μg) were incubated for 10 min at 30°C (in triplicate) in kinase buffer (12.5 mM MOPS pH 7.2, 12.5 mM β-glycerophosphate,

7.5 mM $MgCl_2$, 7.5 mM EGTA, 0.05 mM NaF, 2 mM dithiothreitol, 0.5 mM $NaVO_4$) containing a mitogen-activated protein kinase-specific peptide sequence of myelin basic protein (APRTPGGRR) and [γ-^{32}P]ATP. This peptide sequence contains the threonine-97 phosphorylation site for mitogen-activated protein kinase. Reactions were terminated with 40% trichloroacetic acid and spotted onto phosphocellulose (P81) filters. Filters were washed with 0.75% phosphoric acid, in acetone and dried prior to liquid scintillation spectrometry. Phosphate incorporation, measured in the absence of the substrate peptide, was subtracted from values obtained in the presence of substrate to correct for non-specific phosphorylation.

2.6. Sources of reagents

Affinity-purified polyclonal rabbit antibodies recognizing both erk1 and erk2 proteins (K23), ras (F111), and sos (D21) were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Monoclonal mouse anti-human rass GTPase-activating protein (GAP) was purchased from Upstate Biotechnology Incorporated (Lake Placid, NY, USA). The antiphosphotyrosine antibody (PY20) was obtained from Transduction Laboratories (Lexington, KY, USA). Western blotting was performed using the modular minielectrophoresis system from Bio-Rad (Hercules, CA, USA). Nitrocellulose transfer membranes and P81 filters were obtained from Schleicher and Schuell (Keene, NH, USA). Goat serum and horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse antisera were purchased from GIBCO-BRL (Gaithersburg, MD, USA). Protein A sepharose CL-4B was from Pharmacia Biotech (Piscataway, NJ, USA). Mitogen-activated protein kinase-specific peptide sequence of myelin basic protein was from Upstate Biotechnology (Lake Placid, NY, USA). [γ-^{32}P]ATP was obtained from New England Nuclear (Boston, MA, USA). All other reagents were of biotechnology grade and were obtained from standard commercial sources.

2.7. Analysis of data

Quantitative values are presented as means ± standard error. Statistical significance was tested by analysis of variance with a value of $P < 0.05$ being accepted as significant.

3. Results

3.1. Subcellular distribution of erk1 and erk2

Fig. 1 illustrates the expression of erk1 (apparent molecular weight 44 kDa) and erk2 (apparent molecular weight 42 kDa) in membrane and cytosolic fractions from rat uterus. Between the two subcellular fractions studied, approximately 60% of erk protein was detected in cytosolic fractions. Hormonal status of the animal had no significant effect on erk distribution between membrane and

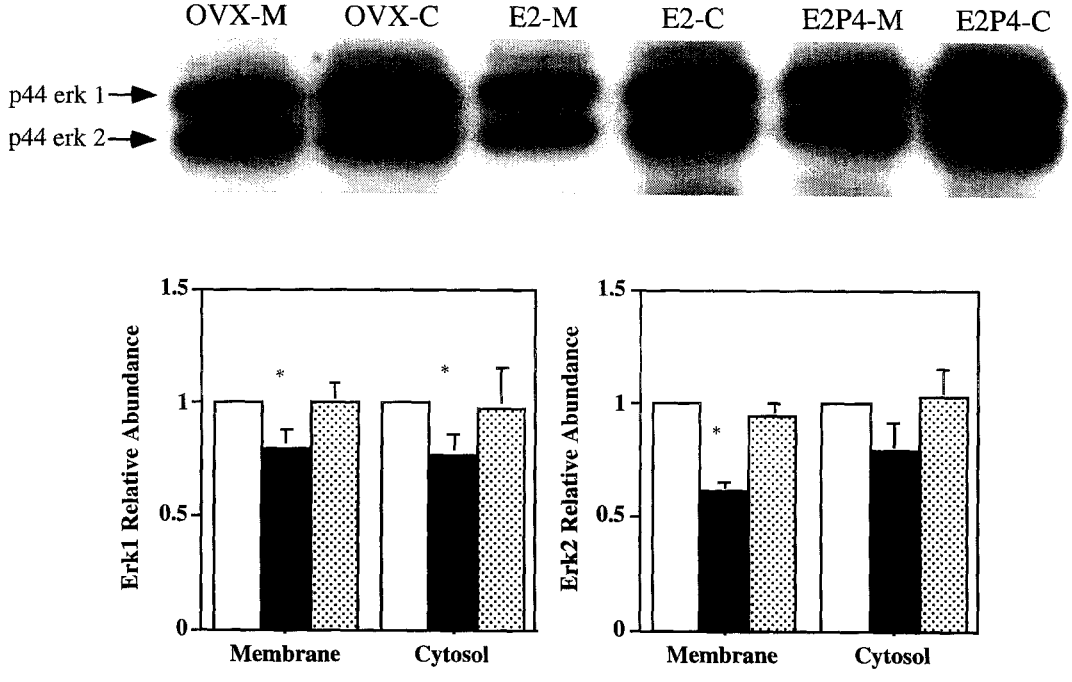

Fig. 1. Top: expression of erk1 (p44) and erk2 (p42) in membrane (M) and cytosolic (C) subcellular fractions in rat uterus from ovariectomized (OVX), estrogen (E2)- and progesterone (E2P4)-treated rats. Uterine subcellular fractions were prepared as described in Methods. Equivalent amounts of protein (35 μg) were loaded onto SDS gels. Bottom: erk1 and erk2 mass is reported as immunoreactive densitometric intensity relative to OVX levels of corresponding subcellular fractions. Results are reported as means ± S.E.M. from three separate experiments. OVX, Open bars; E2, solid bars; E2P4, hatched bars. * Significant difference from OVX.

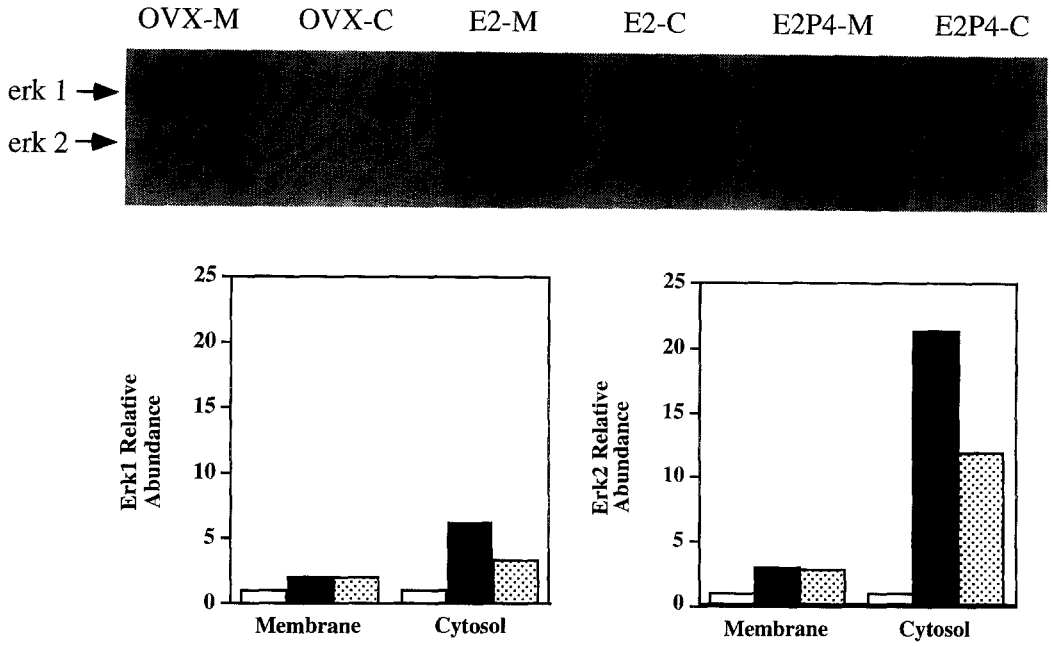

Fig. 2. Top: effect of in vivo estrogen (E2) and progesterone (E2P4) on uterine membrane (M) and cytosol (C) erk tyrosine phosphorylation. Proteins were immunoprecipitated with PY20 antibodies as described in Methods. Equivalent amounts of protein were incubated with anti-phosphotyrosine antibody. Equivalent volumes of precipitated proteins were run on SDS-PAGE and blotted with erk antibodies. Blot shown is representative of a single experiment performed in triplicate. Bottom: erk1 and erk2 tyrosine phosphorylation reported as densitometric intensity relative to OVX levels of corresponding subcellular fractions. OVX, open bars; E2, solid bars; E2P4, hatched bars.

cytosolic compartments. When compared to protein levels in tissue from ovariectomized animals, erk2 protein expression in estrogen-dominated tissues was decreased approximately 40% in membrane fractions ($P = 0.002$). Progesterone domination reversed this effect on membrane-associated erk2 expression ($P = 0.008$). Although qualitatively similar effects were oberved in the cytosolic compartment, the effects were not statistically significant.

Hormonal status of the animal had a modest effect on uterine expression of erk1 protein in membrane and cytosolic compartments. When compared to tissue from ovariectomized animals, erk1 levels in estrogen-dominated tissues was decreased approximately 20% in membrane fractions ($P = 0.059$). Progesterone domination also appeared to reverse this effect of estrogen on membrane-associated erk1 expression although the levels between hormone treatment groups did not achieve statistical signifcance ($P = 0.163$).

3.2. Tyrosine phosphorylation of erk1 and erk2

Phosphorylation of mitogen-activated protein kinases on tyrosine residues by kinases, such as MEK, is associated with increased mitogen-activated protein kinase activity (Blenis, 1993). Phosphorylation of mitogen-activated protein kinases at tyr-185 and thr-183 is required for full enzymatic activation (Davis, 1993). To assess qualitatively whether hormonal status affects basal mitogen-activated protein kinase activity, tyrosine phosphorylation of erk1 and erk2 proteins was investigated. Tyrosine-phospho-

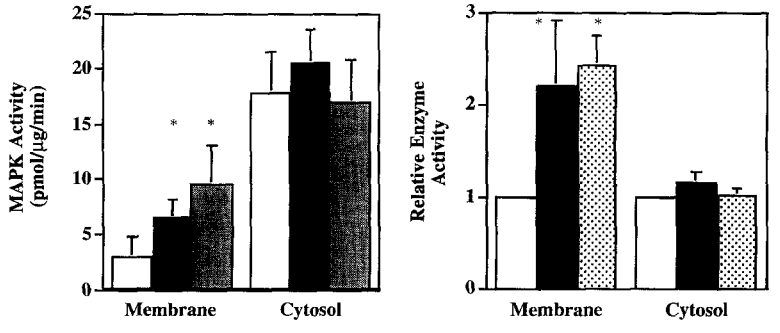

Fig. 3. Comparison of basal MAP kinase enzymatic activity in uterine subcellular fractions from ovariectomized (OVX; open bars), estrogen (E2; solid bars)- and progesterone (E2P4; hatched bars)-treated animals. The results represent the means ± S.E.M. from four independent experiments performed in triplicate. * Significant difference from OVX. Left panel: absolute enzyme activity. Right panel: enzyme activity relative to ovariectomized levels.

rylated proteins were immunoprecipitated with PY20 antibody from equivalent amounts (35 μg) of subcellular fraction protein. Equivalent volumes of immunoprecipitated samples were resolved using SDS-PAGE and erk proteins immunodetected with erk-specific antibodies.

As shown in Fig. 2, hormonal status had distinct effects on the tyrosine phosphorylation of both erk1 and erk2 proteins. In comparison to control (ovariectomized) tissues, estrogen domination resulted in increased tyrosine phosphorylation of both erk1 (Fig. 2, left panel) and erk2 (Fig. 2, right panel) proteins. The phosphorylation of cytosolic erk proteins was more sensitive to hormonal status than in membrane fractions. Progesterone domination also increased the level of tyrosine phosphorylation of erk1 and erk2 proteins. While the phosphorylation of membrane-associated erk proteins was not different from that observed in estrogen-dominated tissues, cytosolic phosphorylation of erk proteins was approximately half of that found in estrogen-dominated tissues.

3.3. MAPK enzymatic activity

Quantitative assessment of the influence of hormonal status on basal mitogen-activated protein kinase activity was examined by investigating the phosphorylation of a mitogen-activated protein kinase-specific peptide sequence of myelin basic protein. As shown in Fig. 3, steroid treatment was associated with elevated enzyme activity in membrane but not cytosolic fractions of rat uterus. Both estrogen and progesterone domination were associated with significantly increased membrane-associated mitogen-activated protein kinase activity. No significant differences in mitogen-activated protein kinase activity were observed between fractions from estrogen- or progesterone-dominated tissues.

3.4. Membrane expression of ras and ras activity-regulating proteins, sos and GAP

The initial transduction steps of mitogenic and hypertrophic stimuli signaling via mitogen-activated protein kinases are thought to involve the activation of a small GTP-binding protein, ras (Seger and Krebs, 1995). The activation of ras is controlled by several chaperone proteins which regulate the rates of GTP binding and hydrolysis by ras. The ras-specific guanine nucleotide exchange factor, sos, activates ras by stimulating the exchange of GDP for GTP (Aronheim et al., 1994). Ras proteins are then deactivated by interaction with specific GTPase-activating proteins, rasGAP that promote GTP hydrolysis by ras (Polakis and McCormick, 1993). To assess qualitatively whether steroid-dependent basal mitogen-activated

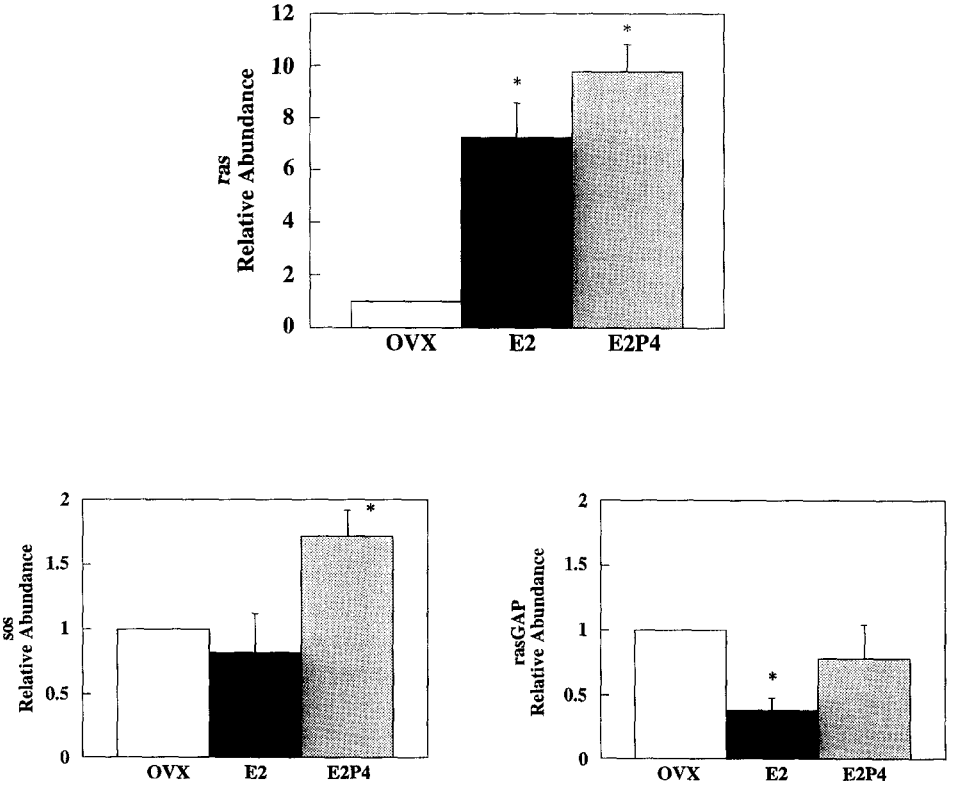

Fig. 4. Expression of ras (top), sos (left), and rasGAP (right) in membrane subcellular fractions in rat uterus from ovariectomized (OVX), estrogen (E2)- and progesterone (E2P4)-treated rats. Uterine subcellular fractions were prepared as described in Methods. Equivalent amounts of protein (35 μg) were loaded onto SDS gels. Protein is reported as immunoreactive densitometric intensity relative to OVX levels of corresponding subcellular fractions. Results are reported as means ± S.E.M. from three separate experiments. OVX, open bars; E2, solid bars; E2P4, hatched bars. * Significant difference from OVX.

protein kinase activity could be related to up-regulation of ras or ras activity, the relative membrane expression of ras, sos, and rasGAP was investigated.

As shown in Fig. 4, membrane content of ras protein was significantly elevated in uteri of estrogen (7.24 ± 1.35-fold)- and progesterone (9.76 ± 1.06)-dominated animals relative to levels in control ovariectomized animals. Membrane levels of sos (0.82 ± 0.30) were not significantly different in estrogen-dominated tissues relative to control levels. However, the effect of progesterone domination resulted in significant up-regulation of membrane levels of sos (1.72 ± 0.20) relative to control tissues. In contrast, rasGAP levels were significantly decreased (0.38 ± 0.10) in estrogen-dominated tissues relative to control levels.

To qualitatively assess the relative potential for ras activition, the ratio of sos : rasGAP levels was examined in all three groups. A ratio of > 1.0 was taken to indicate increased potential for ras activation. Conversely, a ratio of < 1.0 was taken to indicate decreased potential for ras activation due to greater levels of deactivating rasGAP levels. Using the ratio of sos and rasGAP levels in ovariectomized tissues as a reference (1.0), the sos : rasGAP ratio was elevated in estrogen (2.16)- and progesterone (2.21)-dominated tissues.

4. Discussion

In this study, the effects of estradiol-17β and progesterone on mitogen-activated protein kinase expression and activity have been examined. It has long been recognized that these steroid hormones exert profound changes on the uterus, including cellular hypertrophy and changes in uterine contractility (Challis and Mitchell, 1981).

Tyrosine phosphorylation of cellular proteins is recognized as a critical process leading to diverse growth-related responses. Mitogen-activated protein kinases are ubiquitous serine/threonine protein kinases which are acutely activated in responses to growth factors and hormones (Blenis, 1993; Seger and Krebs, 1995). These kinases differ from other common cellular kinases in that they require tyrosine phosphorylation for activation but phosphorylate substrates on serine and threonine residues. Activation of the mitogen-activated protein kinase cascade can occur by direct phosphorylation by growth factor receptors (which contain inherent tyrosine kinase activity), intracellular protein kinase C, and oncogenic small molecular weight GTP-binding proteins such as ras (Hall, 1994). In addition, mitogen-activated protein kinases are capable of autophosphorylation (Seger and Krebs, 1995).

Mitogen-activated protein kinases, when activated, translocate to different cellular compartments (plasma membrane and nucleus) affecting protein function and gene expression associated with cellular hypertrophy and smooth muscle contraction. Mitogen-activated protein kinases are thought to associate with cell membranes to phosphorylate microtubule-associated proteins affecting muscle contractility and cytoskeletal orientation (Chen et al., 1994; Khalil and Morgan, 1994; Pavalko et al., 1995).

In this study, short term pharmacologic hormone replacement regimens in ovariectomized animals were used to provide a parallel to endocrine environments similar to that found during pregnancy (progesterone-dominated) and parturition (estrogen-dominated). Using these regimens, the well described steroid-mediated uterine smooth muscle hypertrophy was observed. In both conditions, estrogen- and progesterone-dominated, mitogen-activated protein kinase tyrosine phosphorylation and activity was elevated when compared to the absence of steroid treatment. These findings are consistent with previous reports of increased mitogen-activated protein kinase activity associated with arterial (Langan et al., 1994) and tracheal smooth muscle proliferation and hypertrophy (Abe et al., 1994). Similarly, cardiac muscle cell hypertrophy is associated with elevated mitogen-activated protein kinase activity (Thorburn et al., 1994).

Interestingly, estrogen down-regulated erk expression in the membrane compartment even though mitogen-activated protein kinase-like enzyme activity and erk tyrosine phosphorylation was elevated. These findings suggest that estrogen may also exert effects on the expression or activity of upstream regulators of mitogen-activated protein kinases. Alternatively, estrogen may facilitate translocation of these proteins to the nuclear compartment. The down-regulation of erks appears to be steroid-specific as progesterone treatment returned proteins to pretreatment levels.

The 2-fold increase in membrane-associated mitogen-activated protein kinase-like activity with steroid treatment may be due to localization of these proteins near upstream regulators. As the upstream regulators of mitogen-activated protein kinase, such as ras, raf-1, and protein kinase C, are resident in the membrane when activated, this finding was not entirely surprising. The localization of mitogen-activated protein kinase with both upstream effectors and the localization and phosphorylation of mitogen-activated protein kinase cytoskeletal susbtrates (e.g. microtubule-associated protein, talin, paxillin) and contractile protein (e.g. caldesmon) substrates has been reported to occur during physiologic smooth muscle contractions (Khalil and Morgan, 1994; Walsh et al., 1994; Pavalko et al., 1995). Of note, uterine contractility is increased under the estrogen-dominated conditions where membrane-associated mitogen-activated protein kinase activity is elevated.

The mechanism for the steroid-associated increase in mitogen-activated protein kinase tyrosine phosphorylation and activity is currently unknown. Mitogen-activated protein kinases are phosphorylated through a signaling cascade of GTPases and protein kinases linking the cell surface to the nucleus (Blenis, 1993; Davis, 1993, Seger and Krebs, 1995). Thus steroid-dependent regulation of upstream regulators of mitogen-activated protein kinase

phosphorylation may in part be involved. Many cell surface mitogenic signals are transduced intracellularly by the GTPase, ras (Hall, 1994). In this study, uterine membrane levels of ras protein were observed to be susbstantially elevated in hormone-dominated tissues exhibiting hypertrophy and elevated mitogen-activated protein kinase activity. Similarly, the ratio of ras stimulatory to deactivating protein levels (sos : rasGAP) was elevated in estrogen- and progesterone-dominated tissues suggesting that up-regulation of ras may be associated with increased ras signaling activity. Targeting of the guanine nucleotide exchange factor, sos, to the membrane in the vicinity of ras appears to be the primary mechanism leading to the activation of the ras pathway in NIH 3T3 cells (Aronheim et al., 1994).

Ras recruits the serine/threonine protein kinase, raf-1, to the cell membrane where raf-1 becomes phosphorylated and in turn, phosphorylates mitogen-activated protein kinase kinases, termed MEK (Avruch et al., 1994). The serine/threonine protein kinase, raf-1, has been described to be important for progesterone-induced mitogen-activated protein kinase activation and cell cycle regulation in *Xenopus* oocytes (Muslin et al., 1993). In C7 3T3 cells, estrogen-dependent raf-1 activates mitogen-activated protein kinase and cellular transformation (Samuels et al., 1993). However, although activation of the estrogen receptor by estradiol increased mitogen-activated protein kinase activity, raf-1 activity was unchanged suggesting the requirement of other factors (perhaps MEK) in the activation of mitogen-activated protein kinase. Other studies indicate that steroid activation of mitogen-activated protein kinase may involve other intermediate protein kinases besides raf-1, such as mos, similarly regulating mitogen-activated protein kinase kinase (MEK) activity (Carnero et al., 1994). Alternatively, steroid hormones may regulate the activity of mitogen-activated protein kinase-specific protein phosphatases (Nebreda, 1994).

The lack of a clear correlation between steroid-dependent mitogen-activated protein kinase phosphorylation and activity in uterine subcellular fractions may be due to several factors. Phosphorylation of the MAPK peptide sequence of myelin basic protein is presumably an index of composite enzyme activity of many mitogen-activated protein kinases, including erk1 and erk2. Thus, the enzyme activity detected may be more appropriately labeled mitogen-activated protein kinase-like. If the uterine subcellular fractions contain substantial amounts or activities of other mitogen-activated protein kinases, such as erk3 or jnk (Seger and Krebs, 1995), then the assay of myelin basic protein peptide phosphorylation by either erk1 or erk2 would be largely masked. The assay of enzyme activity also reflects compartmentalization of different mitogen-activated protein kinase activators and substrates. Increased membrane-associated mitogen-activated protein kinase activity was associated with elevated mitogen-activated protein kinase tyrosine phosphorylation in estrogen- and progesterone-dominated tissues. However, the degree of tyrosine phosphorylation of erk proteins was much smaller than that of enzyme activity. This may be a result of phosphotyrosine phosphatase activity or the presence of binding proteins which further mask mitogen-activated protein kinase enzyme activity. Additionally, direct correlation of tyrosine phosphorylation of mitogen-activated protein kinases with enzyme activity is incomplete as full enzyme activity requires additional phosphorylation of the threonine-183 residue of the proteins.

Although hormonal status altered cytosolic mitogen-activated protein kinase tyrosine phosphorylation, enzyme activity was unaffected. These findings indicate that cytosolic mitogen-activated protein kinase tyrosine phosphorylation may not be a reliable indicator of enzyme activity and that phosphorylation of mitogen-activated protein kinase threonine residues may be more important in this cellular compartment. Although the cytosolic substrates of mitogen-activated protein kinase are not completely known, mitogen-activated protein kinase is thought to translocate to the nucleus and directly bind to transcription factors such as c-myc, c-fos, and c-jun (Blenis, 1993; Davis, 1993). The binding of mitogen-activated protein kinase to c-myc does not require erk2 enzyme activity, suggesting that the mitogen-activated protein kinase-transcription factor complex represents an intermediate in the phosphorylation reaction rather than a non-productive enzyme-substrate reaction (Gupta and Davis, 1994).

In conclusion, the present studies have established that estrogen and progesterone increase the activity of mitogen-activated protein kinases in specific subcellular compartments. The effect of these steroids in the uterine membrane compartment is associated with increased levels of ras and perhaps ras signaling. That regulation of membrane-associated mitogen-activated protein kinase activity by steroid hormones may account for the altered uterine reactivity associated with altered hormonal status requires further study.

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