

Divergent impact of progesterone and medroxyprogesterone acetate (Provera) on nuclear mitogen-activated protein kinase signaling

Jon Nilsen and Roberta Diaz Brinton*

Department of Molecular Pharmacology and Toxicology and Program in Neuroscience, Pharmaceutical Sciences Center, University of Southern California, Los Angeles, CA 90089

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The impact of progestins on estrogen-inducible mechanisms of neuroprotection was investigated. Previously, we showed that estrogen and progesterone are neuroprotective against excitotoxicity, whereas the synthetic progestin medroxyprogesterone acetate (MPA; Provera) is not. Here, we demonstrate that 17 β -estradiol (E₂) and progesterone (P₄) treatment of hippocampal neurons attenuated the excitotoxic glutamate-induced rise in intracellular calcium concentration. Although MPA had no effect alone, MPA completely antagonized E₂-induced attenuation of intracellular calcium concentration. Activation of extracellular receptor kinase (ERK) is required for estrogen-induced neuroprotection and calcium regulation. Paradoxically, E₂, P₄, and MPA all elicited similar rapid and transient activation of ERK, presenting a contradiction between the dependence on ERK for gonadal hormone-induced neuroprotection and the lack of neuroprotection induced by MPA. Subcellular analysis of ERK demonstrated that the phospho-ERK signal is transduced to the nucleus only by E₂ and P₄, not by MPA. These results indicate that the profile of nuclear translocation of ERK is consistent with the neuroprotective profile. Further, the E₂-induced nuclear translocation of ERK was blocked by coadministration of MPA. Results of this study reveal that nuclear ERK induction by ovarian steroids is predictive of the neuroprotective effects of estrogen and progestin treatments, revealing a hitherto unrecognized divergence of progestin signaling through the src/MAPK pathway. These results have much broader implications encompassing the impact of progestins on estrogen-mediated effects in multiple tissues. The recent results from the Women's Health Initiative trial, which used MPA as the progestinal agent, indicate that differences between progestin formulations are crucial to health outcomes in women.

Neurologic benefits of estrogen replacement therapy in humans include reversal of estrogen deficiency-induced memory dysfunction and reduced risk of Alzheimer's disease (AD) (1–5). Recently, the Cache County Study confirmed a reduced risk of AD in elderly women on hormone replacement therapy (HRT) (6). Because progestins are added to HRT to prevent hyperplasia of the endometrium (7) and resulting uterine cancer (8), possible impacts of progestins need to be determined. Numerous studies have found contradictory effects of estrogen/progestin use and breast cancer (9), coronary heart disease (10), and cognition (11, 12). Although some of these studies used the same hormone formulation [conjugated estrogens (Premarin) with medroxyprogesterone acetate (MPA; Provera)], many did not determine or subdivide the type of progestin used (9–14), raising the possibility that the apparent discrepancies in outcomes are due to the differences in cellular responses induced by different progestins. Such concerns have been underscored by the termination of the combined regimen arm of the Women's Health Initiative trial (11, 12, 15).

In earlier work, we found that 17 β -estradiol (E₂) and progesterone (P₄), but not MPA, exerted neuroprotection against glutamate neurotoxicity (16). Not only was MPA an ineffective neuroprotectant, it attenuated E₂-induced neuroprotection

when coadministered (16). Estrogen-induced neuroprotection against excitotoxic glutamate is correlated with an attenuated rise in intracellular calcium concentration ([Ca²⁺]_i) (17, 18). Thus, we sought to determine the impact of P₄ and MPA on excitotoxic glutamate-induced [Ca²⁺]_i rise. Here we show that E₂ and P₄ attenuated the glutamate-induced rise in [Ca²⁺]_i. Although MPA had no effect on the glutamate-induced Ca²⁺ signal, it blocked E₂-induced attenuation.

Although the full underlying cascade of mechanisms remains unidentified, the mitogen-activated protein kinase (MAPK) cascade is required for estrogen-mediated neuroprotection (19) and estrogen-induced attenuation of the [Ca²⁺]_i rise (17). E₂, P₄, and MPA all activate p44/p42MAPK [extracellular receptor kinase (ERK)] (16), yet only E₂ and P₄ are neuroprotective (16). To resolve the paradox between dependence on MAPK for gonadal hormone-induced neuroprotection and lack of neuroprotection induced by some progestins that activate MAPK, we analyzed the temporal and subcellular profile of ERK activation by E₂, P₄, and MPA. We show that E₂ and P₄ rapidly and transiently activated nuclear ERK in hippocampal neurons. In contrast, ERK activated by MPA remained cytosolic with no nuclear signal. Further, MPA blocked the E₂-induced nuclear ERK activation. The dramatic differences in signaling elicited by P₄ and MPA indicate that all progestins are not alike in their induction of cellular responses and, hence, health outcomes.

Materials and Methods

Chemicals. Culture materials were from GIBCO/BRL. Chemicals were from Sigma, unless otherwise noted. Steroids were dissolved in ethanol and diluted in culture medium with final ethanol concentration <0.001%. Fura 2-AM (the acetoxymethyl ester) was from Molecular Probes.

Neuronal Culture. Hippocampal neurons from embryonic day 18 (E18) rat fetuses were cultured as described and generated cultures 98% neuronal in phenotype (17). In brief, embryonic rat hippocampi were dissociated by passage through fire-polished-constricted Pasteur pipettes. Neurons plated on poly-D-lysine-coated coverslips (22-mm-diameter round), four-well chamber slides (Falcon), or polyethylenimine-coated six-well plates were grown in Neurobasal medium (GIBCO/BRL) supplemented with 5 units/ml penicillin, 5 mg/ml streptomycin, and B27 supplement at 37°C in humidified 5% CO₂/95% air atmosphere for 10–12 days before experimentation.

Measurement of Cytoplasmic Ca²⁺ by Using Fura 2-AM. Hippocampal neurons were treated with E₂ (10 ng/ml), P₄ (10 ng/ml), and/or MPA (10 ng/ml) or vehicle control for 48 h before loading in the

Abbreviations: AD, Alzheimer's disease; [Ca²⁺]_i, intracellular calcium concentration; E₂, 17 β -estradiol; P₄, progesterone; HRT, hormone replacement therapy; MPA, medroxyprogesterone acetate; MAPK, mitogen-activated protein kinase; ERK, extracellular receptor kinase; pERK, phospho-ERK; DAPI, 4',6-diamidino-2-phenylindole.

*To whom correspondence should be addressed. E-mail: rbrinton@hsc.usc.edu.

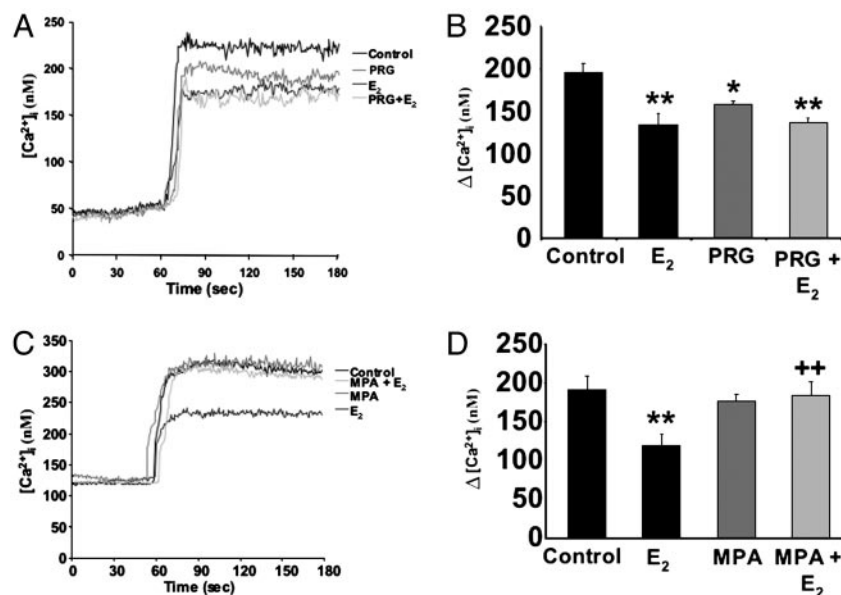


Fig. 1. E₂ and P₄, but not MPA, attenuate the excitotoxic glutamate-induced rise in [Ca²⁺]_i. Hippocampal neurons pretreated with E₂ (10 ng/ml), P₄ (PRG), or both exhibited a significantly lower response to glutamate (200 μM) than control neurons. MPA had no effect on glutamate-induced Ca²⁺ signaling, but blocked the estrogen-mediated attenuation. (A) Representative tracings of the average [Ca²⁺]_i from 10 neurons over time in response to glutamate in the presence or absence of P₄ and/or E₂. (B) Quantitative changes in [Ca²⁺]_i in response to glutamate in the presence or absence of P₄ and/or E₂. (C) Representative tracings of the average [Ca²⁺]_i from 10 neurons over time in response to glutamate in the presence or absence of MPA and/or E₂. (D) Quantitative changes in [Ca²⁺]_i in response to glutamate in the presence or absence of MPA and/or E₂. *, *P* < 0.05 vs. control neurons; **, *P* < 0.01 vs. control neurons; ++, *P* < 0.01 vs. E₂-treated neurons; *n* = 4 independent experiments with ≥10 neurons per experiment.

dark with fura 2-AM (2 μM) in Hanks' balanced salt solution (45 min; 37°C). [Ca²⁺]_i was determined by comparing the A₃₄₀/A₃₈₀ ratio to a standard curve as described (17). Data are presented as representative traces averaged from at least 10 cells per coverslip. Responses to steroids were quantified as the difference between the average [Ca²⁺]_i for 1 min during glutamate exposure (30–90 sec after glutamate exposure) and the average [Ca²⁺]_i for 1 min before exposure. Changes in [Ca²⁺]_i are presented as mean ± SEM from four independent experiments with at least 30 cells per experiment. Equal dye loading was determined as described (18).

Western Blotting. Immunoblottings of whole-cell lysates and subcellular fractions were performed with Anti-Active MAPK antibody (1:750 in PBS-T/1% horse serum; Cell Signaling Technologies, Beverly, MA; PBS-T is PBS containing Tween) or total ERK2 antibody (C-14) (1:5,000 in PBS-T/1% horse serum; Santa Cruz Biotechnology) as described (16). Cytosolic and nuclear proteins were prepared by differential centrifugation. Cell pellets resuspended in 5 vol of cytoplasmic extract (CE) buffer [10 mM Hepes (pH 7.5)/60 mM KCl/1 mM EDTA/1 mM DTT/1 mM sodium orthovanadate/1 mM PMSF/0.075% (vol/vol) Igepal CA-630, Sigma] were homogenized by repeated passage through a 23-gauge needle. Homogenate was centrifuged at 500 × *g* for 5 min at 4°C to pellet out nuclei. Supernatants were centrifuged at 10,000 × *g* for 10 min at 4°C. The resulting supernatants were used as cytoplasmic extracts. Nuclear pellets were washed with 100 μl of CE buffer and resuspended in 1 vol of nuclear extract (NE) buffer [20 mM Tris·HCl/420 mM NaCl/60 mM KCl/1 mM EDTA/1 mM sodium orthovanadate/1 mM PMSF/25% (vol/vol) glycerol; pH 8.0]. Salt concentration was adjusted to 400 mM by addition of 5 M NaCl, followed by addition of 1 vol of NE buffer. Nuclear extracts were incubated for 10 min at 4°C and centrifuged at 10,000 × *g* for 10 min at 4°C. The resulting supernatants were used as nuclear extracts. Specificity of subcellular fractionations

was determined by probing parallel Western blots with antihistone (nuclear) and anti-neuron-specific enolase (cytoplasm).

Immunocytochemistry. Hippocampal neurons exposed to E₂ (10 ng/ml), P₄ (10 ng/ml), or MPA (10 ng/ml) for 30 min were paraformaldehyde (4%)-fixed and Triton X-100 (0.001%)-permeabilized before incubation with anti-Active MAPK antibody (1:300; Cell Signaling Technologies) followed by incubation in fluorescein-conjugated goat anti-rabbit secondary antibody (1:250; Vector Laboratories) for 45 min at RT. Cells were mounted in Vectashield mounting medium with 4',6-diamidino-2-phenylindole (DAPI) (Vector Laboratories). Relative immunoreactive intensity was calculated by using INCYTIM1 software (Intracellular Imaging, Cincinnati). The area of DAPI staining was mapped to the FITC images to define the nucleus as the region of interest or as a mask to define the cytoplasm as the region of interest. The relative fluorescence intensities of 20 randomly selected cells were normalized to the average fluorescence intensity of control cells and presented as mean ± SEM. The cytoplasm and nucleus were analyzed independently of each other.

Statistics. Statistically significant differences between groups were determined by an ANOVA followed by a Newman-Keuls post hoc analysis.

Results

E₂ and P₄ Attenuate the Glutamate-Induced Rise in Intracellular Calcium. Estrogen-induced neuroprotection against excitotoxic glutamate is correlated with an attenuated rise in [Ca²⁺]_i (17, 18). Thus, we sought to determine the impact of P₄ and MPA on excitotoxic glutamate-induced [Ca²⁺]_i rise. Accordingly, our first aim was to compare the rise in [Ca²⁺]_i, as measured by Fura2, in E₂-, P₄-, and MPA-treated neurons.

Glutamate induced a rapid increase in [Ca²⁺]_i (Fig. 1) with a mean change in [Ca²⁺]_i obtained on stimulation with 200 μM

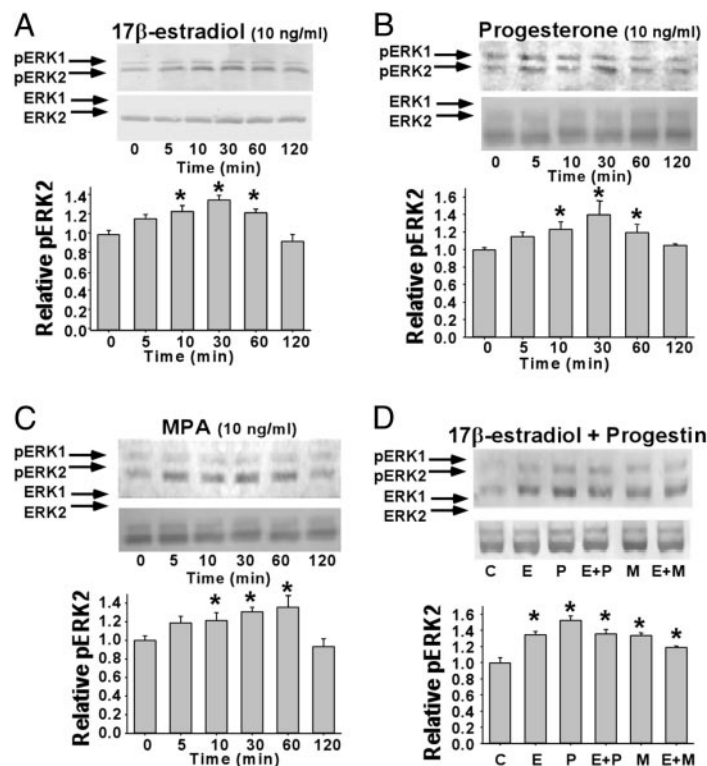


Fig. 2. Rapid activation of ERK-2 in primary hippocampal neurons treated with E₂, P₄, or MPA. Western blots show levels of pERK2 and total ERK2 in whole-cell lysates from primary hippocampal neurons treated with E₂ (A), P₄ (B), MPA (C), or combined E₂ and progestin (D). C, vehicle; E, E₂; P, P₄; M, MPA. Bar graphs represent mean \pm SEM; *, $P < 0.05$ as compared with control; $n = 4$.

glutamate alone of 196.8 ± 10.5 nM ($n = 4$; Fig. 1B). Hippocampal neurons pretreated with E₂ exhibited a marked ($\approx 30\%$) and significant decrease in $[Ca^{2+}]_i$ generated by 200 μ M glutamate (Fig. 1A) with a mean change in $[Ca^{2+}]_i$ of 135.1 ± 10.2 nM ($P < 0.01$; $n = 4$ experiments; Fig. 1B). Likewise, P₄-treated neurons exhibited a marked and significant decrease in $[Ca^{2+}]_i$ generated by 200 μ M glutamate (Fig. 1A) with a mean change in $[Ca^{2+}]_i$ of 158.7 ± 3.6 nM ($P < 0.05$; $n = 4$ experiments; Fig. 1B). The attenuation of the glutamate-induced rise in $[Ca^{2+}]_i$ was non-significantly less with P₄ treatment than with E₂ treatment (Fig. 1B). Hippocampal neurons pretreated with the combination of E₂ (10 ng/ml) and P₄ (10 ng/ml) showed attenuation of the glutamate-induced rise in $[Ca^{2+}]_i$ (Fig. 1A), with a mean change in $[Ca^{2+}]_i$ of 137.1 ± 5.6 nM ($P < 0.01$ as compared with control; $n = 4$; Fig. 1B), an effect more like that of E₂ than P₄.

MPA Blocks the E₂-Induced Attenuation of the Glutamate-Induced Rise in Intracellular Calcium. Hippocampal neurons were exposed to MPA (10 ng/ml) or vehicle control in the presence and absence of E₂ (10 ng/ml) for 48 h before monitoring $[Ca^{2+}]_i$ in response to glutamate (200 μ M). Glutamate induced a rapid increase in $[Ca^{2+}]_i$ (Fig. 1C), with a mean change in $[Ca^{2+}]_i$ of 191.1 ± 17.8 nM (Fig. 1C). Neurons pretreated with E₂ again exhibited a significant attenuation of glutamate-stimulated increase in $[Ca^{2+}]_i$ (Fig. 1C), with a mean change in $[Ca^{2+}]_i$ of 119.2 ± 14.5 nM ($P < 0.01$; $n = 4$; Fig. 1D). Neurons pretreated with MPA exhibited no change in glutamate-stimulated rise in $[Ca^{2+}]_i$ ($n = 4$; Fig. 1C), with a mean change in $[Ca^{2+}]_i$ of 176.9 ± 8.9 ($n = 4$; Fig. 1D). Coadministration of MPA with E₂ significantly antagonized the E₂-induced attenuation of the glutamate-stimulated rise in $[Ca^{2+}]_i$ (Fig. 1C), with a mean change in $[Ca^{2+}]_i$ of 183.5 ± 18.1 nM ($P < 0.01$ as compared with E₂-treated neurons; $n = 4$; Fig. 1D).

MAPK Activation in Response to E₂, P₄, and MPA in Primary Hippocampal Neurons. Blockade of MAPK activation prevents estrogen-mediated attenuation of excitotoxic glutamate-induced rise in $[Ca^{2+}]_i$ (16) and the associated neuroprotection (19). These findings suggest that the ovarian steroid-induced neuroprotection and the attenuation of excitotoxin-induced $[Ca^{2+}]_i$ signaling rise are dependent on active MAPK. Yet MPA, which neither exerts neuroprotection nor alters Ca²⁺ signaling, activates MAPK equally as well as E₂ and P₄ (16). To resolve the paradox between the dependence on MAPK for gonadal hormone-induced neuroprotection and the lack of neuroprotection induced by MPA, we chose to analyze first the temporal nature of ERK activation by E₂, P₄, and MPA, because the duration of MAPK activation can result in different outcomes (20). Using an antibody recognizing the phosphorylated form of p44/p42 MAPK (ERK1/ERK2) as an indicator of activation (21), Western blot analyses indicated that activation of ERK in primary hippocampal neurons occurs rapidly and transiently after treatment with E₂, P₄, and MPA. The kinetics of ERK activation by E₂, P₄, and MPA were similar, with increased immunoreactivity apparent 5 min after treatment and maximal immunoreactive intensity apparent at 30 min, with a return to basal levels by 120 min (Figs. 2A and 1B and C). Treatment with E₂ (10 ng/ml) resulted in a maximal phospho-ERK2 (pERK2) immunoreactivity increase of $\approx 60\%$ at 30 min (Fig. 2A), which is consistent with previous reports (19, 22). Treatment with P₄ (10 ng/ml) or MPA (10 ng/ml) maximally increased pERK2 immunoreactivity by $\approx 123\%$ (Fig. 2B) and $\approx 60\%$ (Fig. 2C), respectively, at 30 min. Coadministration of P₄ or MPA with E₂ for 30 min increased pERK2 immunoreactivity by $\approx 100\%$ (Fig. 2D) and $\approx 75\%$ (Fig. 2D), respectively, compared with vehicle-treated neurons.

Increased Nuclear pERK in Primary Hippocampal Neurons in Response to E₂ and P₄, but Not MPA. Nuclear signaling by many cellular stimuli depends on activation of the MAPK cascade and nuclear

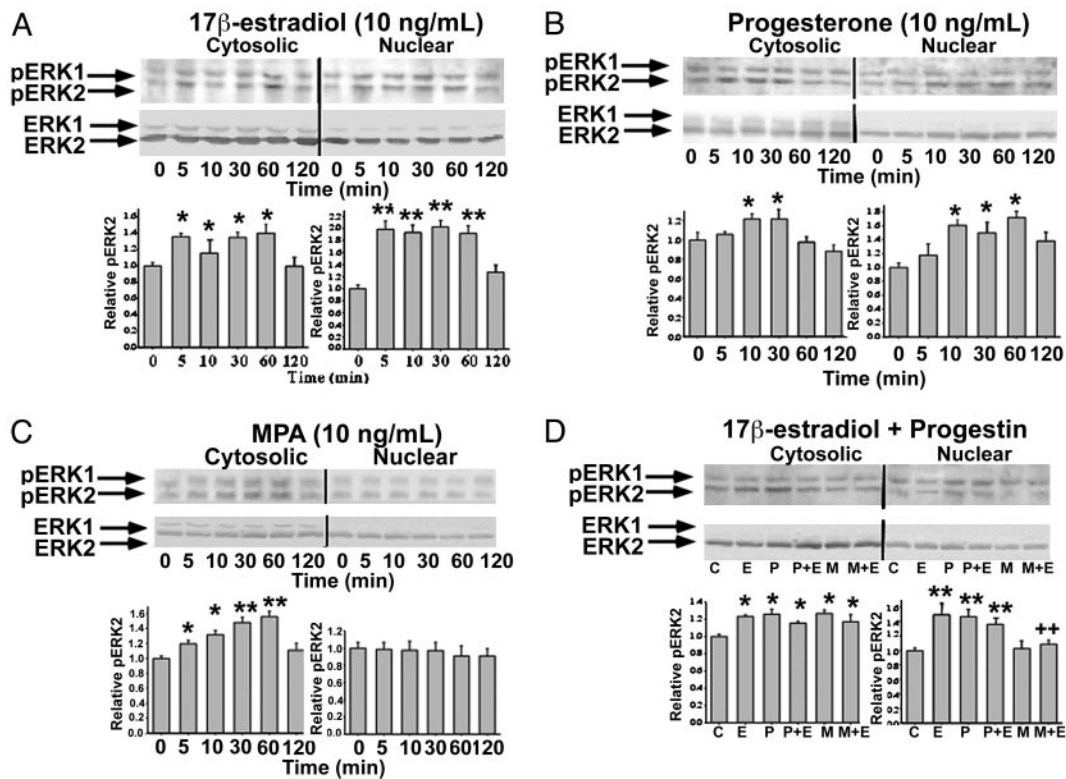


Fig. 3. Rapid activation of nuclear ERK-2 in hippocampal neurons treated with E₂ and P₄, but not with MPA. Western blots show levels of pERK2 and total ERK2 in cytoplasmic and nuclear fractions from primary hippocampal neurons treated with E₂ (A), P₄ (B), MPA (C), or combined E₂ and progesterin (D). Bar graphs represent mean ± SEM; *, *P* < 0.05 as compared with control; **, *P* < 0.01 as compared with control; ++, *P* < 0.01 as compared with E₂ alone; *n* = 4.

localization of active MAPK, where these enzymes can act on their target substrates (23, 24). Such nuclear signaling depends on translocation of MAPK from the cytoplasm to the nucleus (24, 25). To determine whether this critical step was a point of divergence between the progestins, Western blot analysis was performed on cytosolic and nuclear fractions from primary hippocampal neurons treated with E₂, P₄, and MPA (Fig. 3). Results demonstrated that pERK2 immunoreactivity was present at very low levels in both cytosolic and nuclear fractions from control neurons (Fig. 3). In neurons treated with E₂ or P₄, a rapid and transient increase in pERK2 in both cytosolic and nuclear fractions occurred within 5 min (Fig. 3A and B). The kinetics of ERK activation in the cytosolic fraction in response to E₂ and P₄ were similar, with increased immunoreactivity observed at 5 min and maximal staining occurring at 30 min, and immunoreactivity returning to basal levels by 120 min (Figs. 3A and B). Increased immunoreactivity for pERK2 in the nuclear fraction in response to E₂ was observed at 5 min, and maximal staining occurred at 60 min, with immunoreactivity returning to basal levels by 120 min (Fig. 3A). Increased immunoreactivity for pERK2 in the nuclear fraction in response to P₄ was observed at 10 min and maximal staining occurred at 60 min, with a slight decrease in staining intensity at 120 min (Fig. 3B). In contrast to the response to E₂ and P₄, MPA treatment significantly increased pERK2 immunoreactivity in only the cytosolic fraction (Fig. 3C). Increased immunoreactivity was observed at 5 min and maximal staining occurred at 60 min with a return to basal levels by 120 min (Fig. 3C). No detectable increase in pERK2 immunoreactivity occurred in the nuclear fraction in response to MPA treatment at any of the times examined (Fig. 3C).

Coadministration of P₄ or MPA with E₂ resulted in a significant increase in pERK2 immunoreactivity in the cytosolic fraction (Fig. 3D). Coadministration of P₄ with E₂ resulted in a

significant increase in pERK2 immunoreactivity in the nuclear fraction that was similar to that seen for either steroid alone (Fig. 3D). Coadministration of MPA with E₂ completely blocked the increased pERK2 immunoreactivity in the nuclear fraction seen with E₂ alone (Fig. 3D).

Intracellular Distribution of pERK After E₂, P₄, or MPA Treatment of Hippocampal Neurons. To verify the differential pattern of pERK localization observed with Western blot analysis, immunostaining of primary hippocampal neurons was performed to visualize the subcellular distribution of pERK. Untreated control neurons showed weak immunoreactivity for the active form of ERK, which was restricted to the cytoplasm (Fig. 4A). pERK immunofluorescence increased after 30-min treatment with E₂, P₄, or MPA, with staining intensity varying from cell to cell (Fig. 4). In estrogen-responsive neurons, immunoreactive pERK was distributed throughout the cell, appearing in cytoplasm, neuronal processes, and nucleus (Fig. 4B) with a significant increase in pERK immunoreactivity occurring in the nucleus (Fig. 4B). Treatment with P₄ also resulted in increased pERK in the nuclear compartment of the neuron (Figs. 4C and 5B). Although MPA treatment resulted in increased staining intensity (Figs. 4E and 5A), pERK immunoreactivity was located cytoplasmically in MPA-treated cells with nuclei devoid of pERK immunoreactivity (Figs. 4E and 5B).

Coadministration of P₄ with E₂ increased the intensity of pERK immunoreactivity in the cytoplasm and nucleus as compared with baseline levels (Figs. 4D and 5). Coadministration of MPA with E₂ for 30 min increased pERK immunoreactivity, but it restricted the localization of the increased immunoreactive signal to the cytoplasm, which is a pattern of pERK similar to that seen with MPA alone (Figs. 4F and 5).

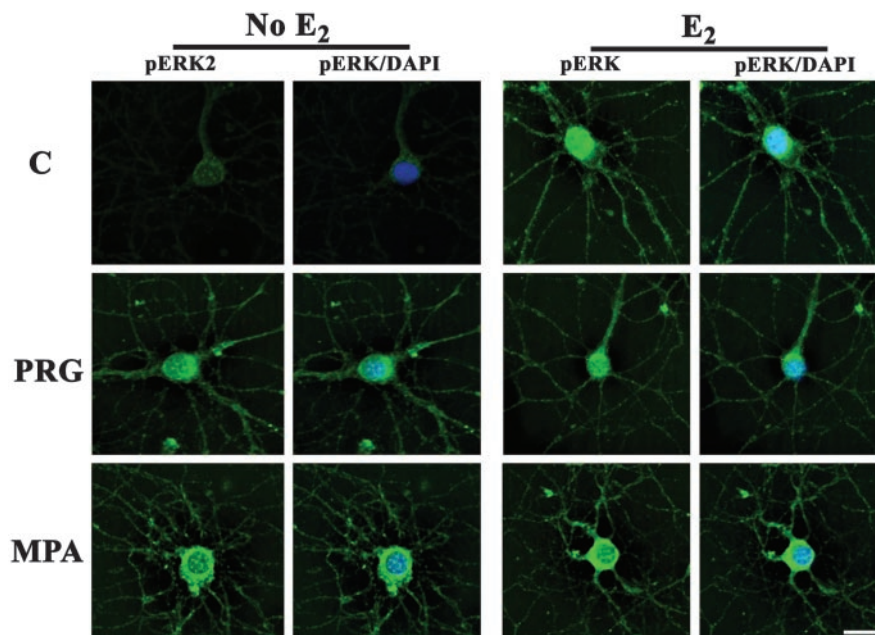


Fig. 4. Nuclear localization of pERK in hippocampal neurons induced by E₂ or P₄, but not MPA. pERK (green; FITC) immunofluorescence and DAPI nuclear counterstain (blue) in primary hippocampal neurons treated with vehicle (C, Top Left), E₂ (Right), P₄ (PRG, Middle), and MPA (Bottom). (Bar, 10 μm.)

Discussion

We demonstrate that different progestins can induce divergent neural responses directly and regulate E₂-mediated regulation of calcium signaling and nuclear activation of ERK. Relevance of these effects for neural survival is predicated on a mechanistic pathway leading to E₂-inducible neuroprotection. Neuroprotection induced by full estrogen agonists against glutamate-induced excitotoxicity is mediated by shunting of Ca²⁺_i into mitochondria,

leading to attenuation of [Ca²⁺]_i (17, 18, 26). In turn, estrogen-induced attenuation of [Ca²⁺]_i rise depends on MAPK/ERK, because blockade of MAPK activation results in a loss of estrogen-induced neuroprotection (16) and in a loss of estrogen-induced attenuation of excitotoxic glutamate-induced rise in [Ca²⁺]_i (19). These data indicate that activation of MAPK is required for the attenuation of excitotoxic glutamate-induced rise in [Ca²⁺]_i that leads to an increase in neuronal survival. Here we show that, like E₂, P₄ attenuates the excitotoxic glutamate-induced rise in [Ca²⁺]_i. In contrast, not only was MPA ineffective at altering glutamate-mediated Ca²⁺ signaling alone, MPA blocked E₂-induced attenuation. Paradoxically, however, MPA, like E₂ and P₄, activated MAPK (16), yet was ineffective at neuroprotection and attenuation of excitotoxic glutamate-mediated Ca²⁺ signaling. These data indicate that ERK activation *per se* is not predictive of neuroprotection, presenting a paradox of the MAPK requirement for steroid-induced neuroprotection.

We sought to resolve the paradox between the dependence of MAPK activation for gonadal hormone-induced neuroprotection and the lack of neuroprotection induced by MPA that also activated ERK. Results of Western blot analysis and immunocytochemistry demonstrated that all three steroids elicited similar rapid and transient activation of ERK. Thus, the divergence between P₄ and MPA must occur downstream of MAPK activation. Building on the findings of Singh and colleagues (22, 27) showing E₂-induced nuclear translocation of ERK, we determined whether E₂, P₄, and MPA induced nuclear translocation of ERK. Surprisingly, the pERK signal was transduced to the nucleus only by E₂ and P₄, not by MPA. Further, E₂-induced nuclear translocation of pERK was blocked by coadministration of MPA. These data indicate that translocation of ERK to the nucleus is a pivotal and necessary requirement for gonadal hormone protection of neurons against excitotoxic insults associated with neurodegenerative disease. A probable downstream consequence of failing to translocate ERK to the nucleus is to prevent E₂ activation of CREB, which is MAPK-dependent (28), and to thwart E₂-induced increase in the antiapoptotic protein Bcl-2. This postulate is consistent with previous findings that E₂

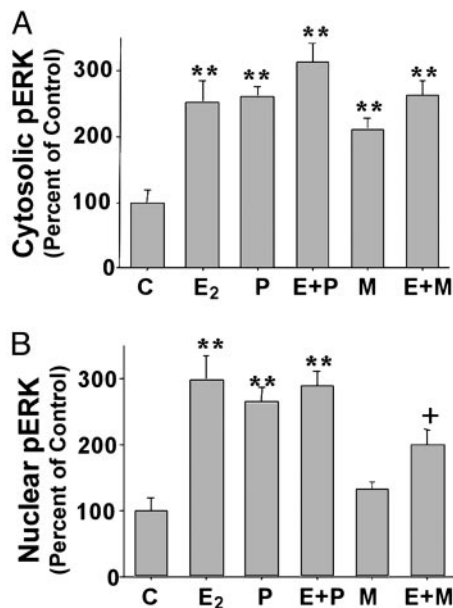


Fig. 5. Subcellular compartmentalization of the fluorescent intensity of the pERK signal is altered by E₂, P₄, and MPA. Bar graphs represent relative fluorescence intensities for pERK localized in cytoplasm (A) and nucleus (B) of primary hippocampal neurons treated with vehicle (C), E₂ (E), P₄ (P), and MPA (M). Data are presented as mean ± SEM; **, *P* < 0.01 as compared with control; +, *P* < 0.05 as compared with E₂ alone; *n* = 20 neurons.

and P₄ induced Bcl-2 expression and MPA blocked E₂-induced Bcl-2 expression (16). The divergence between the induction of ERK nuclear translocation offers a plausible predictive mechanism by which MPA fails to protect neurons against toxic insults and antagonizes E₂-induced neuroprotection and predicts the neuroprotective efficacy of HRT.

Mechanisms underlying the divergent ERK translocation remain to be determined but may include different steps in initiating ERK signaling, differential activation of regulators of ERK nuclear translocation, or activation of distinct pools of MAPK. Several events cooperatively determine the amount of nuclear ERK, such as cytoplasmic anchoring, phosphorylation, and subsequent dimerization, active transport of ERK across the nuclear membrane, and retention in the nucleus (29, 30). Interference at any step by MPA could prevent nuclear translocation of ERK. E₂-induced nuclear translocation of pERK can be blocked by protein synthesis inhibitors (27), implicating an active process, which could be antagonized by MPA. Alternatively, spatial organization of kinases and substrates can determine the transmission and target site of action, providing a localization strategy whereby distinct populations of MAPK can restrict activation of downstream targets (23, 31, 32). Activation of a nontranslocated pool of MAPK could lead to inactivation of proteins responsible for cell survival. Predictive relationships between nuclear pERK and neuroprotective effectiveness of sex steroids suggests a requirement for transcriptional activation (33–35).

The use of HRT as a protective agent against age-related cognitive decline and AD has been supported by the recent Cache County Study (6) and numerous epidemiological retrospective and prospective analyses (for review see ref. 36), which demonstrate significant reduction in incidence and risk of AD with HRT use. In striking contrast, analysis from the Women's Health Initiative Memory Study clinical trial reported that

estrogen plus progestin therapy did not improve cognitive function in older women and resulted in a doubling of the diagnosis of AD (12). The formulation of HRT used in the Women's Health Initiative Memory Study was a combination of MPA and conjugated estrogens [Prempro]. The results of our studies (16, 37) indicate that inclusion of MPA would negate the potential beneficial effects of estrogens on cognitive function and prevention of AD and would thus provide a potential explanation for the adverse outcomes of the Women's Health Initiative Memory Study clinical investigation.

Results obtained in neurons reported here and previously (16, 37) are potentially relevant to other tissues. Multiple studies using different hormone formulations have reported contradictory effects of estrogen/progestin use in breast cancer risk (9) and coronary heart disease (10). In light of our findings, discrepancies in outcomes could be, in part, attributable to differences in the cellular responses induced by different progestins. For example, MPA, but not P₄, mitigated E₂ protection against coronary artery vasospasm in rhesus monkeys (38). In addition, the Postmenopausal Estrogen/Progestins Intervention trial, which compared different HRT regimens, found that P₄ induced a more favorable lipid profile when administered with estrogen than did MPA (14). Collectively, these data demonstrate that all progestins are not alike in induction of cellular responses and, hence, health outcomes. Further, our data indicate that the progestin within a HRT formulation will be pivotal for achieving therapeutic benefit to prevent neurodegenerative disease and to sustain cognitive function throughout the menopausal years.

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