

Impact of Progestins on Estrogen-Induced Neuroprotection: Synergy by Progesterone and 19-Norprogesterone and Antagonism by Medroxyprogesterone Acetate

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Estrogen replacement therapy is associated with improvement of cognitive deficits and reduced incidence of Alzheimer's disease. To compare the impact of therapeutically relevant progestins on estrogen-induced neuroprotection, we treated primary hippocampal neuron cultures with 17β -E2 and progestin, alone and in combination, 48 h before glutamate insult. Estrogen, progesterone, and 19-norprogesterone, alone or in combination, protected against glutamate toxicity. In contrast, medroxyprogesterone acetate (MPA) failed to protect against glutamate toxicity. Not only was MPA an ineffective neuroprotectant but it attenuated the estrogen-induced neuroprotection when coadministered. We addressed the role of MAPK activation in neuroprotection by ovarian ste-

roids. Estrogen and all three progestins tested, alone or in combination, activated MAPK, indicating another mechanism of protection. Bcl-2 expression has been shown to prevent cell death and is up-regulated by 17β -E2. Progesterone and 19-norprogesterone, alone or in combination with estrogen, increased Bcl-2 expression. In contrast, MPA blocked estrogen-induced Bcl-2 expression when coadministered. These results may have important implications for the effective use of hormone replacement therapy in the maintenance of neuronal function during menopause and aging and for protection against neurodegenerative diseases such as Alzheimer's disease. (*Endocrinology* 143: 205–212, 2002)

ALTHOUGH THE RECEPTORS for estrogens and progestins belong to different nuclear receptor subfamilies and recognize distinct hormone response elements, there is considerable cross-talk between the estrogen and progestin signaling pathways. Progestins have been shown to block the estrogen-stimulated expression of both *c-fos* and progesterone receptor mRNAs in uterine cells (1, 2). Furthermore, progestins and antiprogestins have been shown to inhibit estrogen-stimulated uterine proliferation (2, 3). Thus, progestins have been added to hormone replacement therapy (HRT), in part, to reduce the risk of uterine cancers associated with unopposed estrogen (4). Progestins have been added to some formulations of HRT to prevent hyperplasia of the endometrium and tumorigenesis (5). In addition to prevention of endometrial hyperplasia, inclusion of progestins in HRT was thought to reduce estrogenic effects in breast that may be associated with increased risk of breast cancer (6). Contrary to this long-held belief, a recent study found that an estrogen-progestin regimen increased cancer risk beyond that associated with estrogen alone (7). This emphasizes the necessity of studying the modulation of estrogenic effects by progestins in various systems.

It is becoming increasingly apparent that HRT is beneficial in the central nervous system, in part, because of the neurological effects of estrogens, including protection against neurologic insults, reduced risk of Alzheimer's disease (AD),

and improved cognitive function (8, 9). *In vitro* models for these effects have shown that estrogen enhances neuronal survival resulting from oxidative stress, excitotoxic insults, and β -amyloid (10, 11). Although all the mechanisms underlying these effects remain to be identified, recent studies have shown that the tyrosine kinase/MAPK signal transduction cascade may be involved in estrogen-mediated neuroprotection. Estrogen rapidly activated tyrosine kinase and MAPK activity (12–14), and the neuroprotective effect of estrogen against glutamate toxicity was blocked by inhibitors of tyrosine kinase and MAPK (15). The MAPK pathway is thought to play an important role in the actions of neurotrophins, and its activation could lead to increased expression of antiapoptotic genes. One such antiapoptotic gene, bcl-2, is involved in survival of nerve growth factor (NGF)-dependent sensory neurons (16) and inhibits death in response to glutamate toxicity (17). Additionally, Bcl-2 expression is increased in response to estrogen treatment of neuronal cells (18–20).

Very little research on hormonal modulation of neuronal survival has studied the effects of progestins only or progestins in conjunction with estrogen. It is currently unknown what effect different progestins have on estrogen-mediated modulation of MAPK activation and Bcl-2 expression in neuronal cells. It is not clear whether progestins are neuroprotective or not, nor whether they impact the neuroprotective effects of estrogen. Previous studies reported no neuroprotective effect of progestins (21), whereas others have seen positive effects of progestin on neuronal survival (22, 23). Direct comparison between these studies is difficult, because

Abbreviations: AD, Alzheimer's disease; ERT, estrogen replacement therapy; HRT, hormone replacement therapy; LDH, lactate dehydrogenase; MPA, medroxyprogesterone acetate; NGF, nerve growth factor; PBS-Tween, PBS containing 0.05% Tween-20.

each differed in the neuroprotective model studied and in the progestin used.

The purpose of this study was to determine the effects of three widely clinically used progestins [progesterone, 19-norprogesterone, and medroxyprogesterone acetate (MPA)] on a well-established model of estrogen-induced neuroprotection. Results of the present study demonstrate that progesterone and 19-norprogesterone were neuroprotective, alone or when administered in conjunction with 17 β -E2, whereas MPA was not. Not only was MPA an ineffective neuroprotectant, it blocked estrogen-induced neuroprotection when coadministered. Although all three progestins tested activated MAPK, only the neuroprotective progesterone and 19-norprogesterone increased the expression of Bcl-2. These data point out the necessity of examining the composition of hormones used in HRT formulations, especially with regard to which progestin is included. This work belies a common underlying theme in endocrinology, that although structurally different ligands behave the same in some systems, they do not necessarily do so in all systems.

Materials and Methods

Animals

All studies were approved by the University of Southern California Institutional Review Board for animal care. Timed-pregnant Sprague Dawley rats were purchased from Harlan Sprague Dawley, Inc., Indianapolis, IN. They were housed under controlled conditions of temperature (22 C), humidity, and light (14 h light, 10 h dark); and water and food were available *ad libitum*.

Neuronal culture

Primary cultures of dissociated hippocampal neurons were performed as previously described (24). Briefly, hippocampi were dissected from the brains of E18 rat fetuses, treated with 0.02% trypsin in HBSS (50 mM KCl, 3 mM H_2PO_4 , 80 mM NaCl, 0.9 mM $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, 10 mM dextrose, 0.3 M HEPES), for 5 min at 37 C, and dissociated by repeated passage through a series of fire-polished constricted Pasteur pipettes; and 10^6 cells/ml were plated on PEI-coated 96-well plates for toxicity experiments or 6-well plates for immunoblots. Nerve cells were grown in Neurobasal medium (Life Technologies, Inc., Rockville, MD) supplemented with 10 mM NaHCO_3 , 5 U/ml penicillin, 5 mg/ml streptomycin, and B27 supplement (Life Technologies, Inc.). Cultures were maintained at 37 C in a humidified 5% CO_2 atmosphere, and all experiments were performed after 12 d in culture.

Glutamate toxicity

Forty-eight hours before glutamate exposure, cultures were placed in fresh Neurobasal medium supplemented with B27, 10 mM NaHCO_3 , 5 U/ml penicillin, and 5 mg/ml streptomycin, with the steroids as indicated. 17 β -E2 (Sigma), progesterone (Sigma, St. Louis, MO), 19-norprogesterone (Sigma), and MPA (Pharmacia & Upjohn, Inc., Peapack, NJ) were all dissolved in ethanol, to a stock solution of 1 mg/ml; and further dilution was in culture medium so that the final concentration of ethanol was less than 0.01%. Glutamate exposure was performed for 5 min at 37 C in buffer containing 2 mM KCl, 1 mM MgSO_4 , 2.5 mM CaCl_2 , 1 mM NaH_2PO_4 , 4.2 mM NaHCO_3 , 12.5 mM HEPES, 10 mM glucose, 0.1 M NaCl, and 100 μM L-glutamic acid. Cultures were then washed and returned to fresh Neurobasal medium.

Evaluation of plasma membrane damage

Overall neuronal injury was assessed 24 h after the start of the exposure by quantitative measurement of lactate dehydrogenase (LDH) in the bathing medium, an index that is proportional to the total number of neurons damaged by excitotoxic exposure (25). LDH activity was

measured using the Cytotoxicity Detection Kit (Roche Molecular Biochemicals, Indianapolis, IN) and quantitated by measuring the absorbance at 490 nm. Data are normalized against the amount of LDH activity released from vehicle-treated control cultures receiving no glutamate. Data are presented as the mean \pm SEM for at least three independent experiments.

MAPK phosphorylation

Hormones were added to the cultures, as indicated, for 30 min; and the cells were lysed by incubation in ice-cold lysis buffer (0.005% SDS, 0.1% Igepal, 0.2 mM sodium orthovanadate, and 0.2 mM phenylmethylsulfonylfluoride in PBS) for 30 min at 4 C. Cell lysates were cleared by centrifugation at $12,000 \times g$ for 10 min, and the concentration of protein in the supernatant was determined by the BCA Protein Assay (Sigma). Twenty micrograms of total protein from whole-cell lysates were separated under reducing and denaturing conditions by 12% SDS-PAGE and were electrotransferred to polyvinylidene difluoride membrane (Millipore Corp., Bedford, MA). Nonspecific binding sites were blocked with 5% skim milk in PBS containing 0.05% Tween-20 (PBS-Tween). An antibody recognizing the dual threonine and tyrosine phosphorylation sequence from MAPK necessary for activation of the enzyme (antiactive MAPK; Promega Corp., Madison, WI) was used to evaluate ERK1/ERK2 phosphorylation. The membranes were incubated with antiactive MAPK antibody (1:2,000 in PBS-Tween/1% horse serum) or total ERK2 antibody (C-14) (1:5,000 in PBS-Tween/1% horse serum; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) for 1 h. Membranes were then incubated in horseradish peroxidase (HRP)-conjugated horse antimouse IgG (1:10,000), and results were visualized by the TMB Peroxidase Substrate Kit (Vector Laboratories, Inc., Burlingame, CA). Relative amounts of phospho-ERK and total ERK2 were quantitated by optical density analysis using Scion Image software (Scion, Frederick, MD). The level of phospho-ERK was normalized, with respect to total ERK2. So as to avoid interassay variations, the values obtained were also normalized with the value measured for the vehicle-treated control cultures in each experiment. Data are presented as the mean \pm SEM for at least three independent experiments.

Bcl-2 expression

Hormones were added to the cultures, as indicated, for 24 h before collection of whole-cell lysates and SDS-PAGE as above. Blocked membranes were incubated with anti-Bcl-2 antibody (1:250 in PBS-Tween/1% horse serum; Zymed Laboratories, Inc., San Francisco, CA) for 1 h. Membranes were then incubated in horseradish peroxidase (HRP)-conjugated horse antimouse IgG (1:10,000), and results were visualized by the TMB Peroxidase Substrate Kit (Vector Laboratories, Inc.). Relative levels of Bcl-2 were quantitated by optical density analysis using Scion Image software. So as to avoid interassay variations, the values obtained were also normalized with the value measured for the vehicle-treated control cultures in each experiment. Data are presented as the mean \pm SEM for at least three independent experiments.

Statistics

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

Results

Effect of 17 β -E2 on glutamate-induced neurotoxicity

Initial experiments were performed to characterize 17 β -E2's protective effect against glutamate neurotoxicity under the culture conditions used. Neuronal plasma membrane damage was evaluated by measuring LDH release into the media by damaged cells 24 h after glutamate treatment. As previously reported, a 5-min treatment of 100 μM glutamate caused significant cell death (Fig. 1; $P < 0.05$, compared with control; $n = 4$). As expected, 17 β -E2 significantly reduced the amount of LDH released into the media 24 h after exposure to 100 μM glutamate (Fig. 1). The magnitude of neuropro-

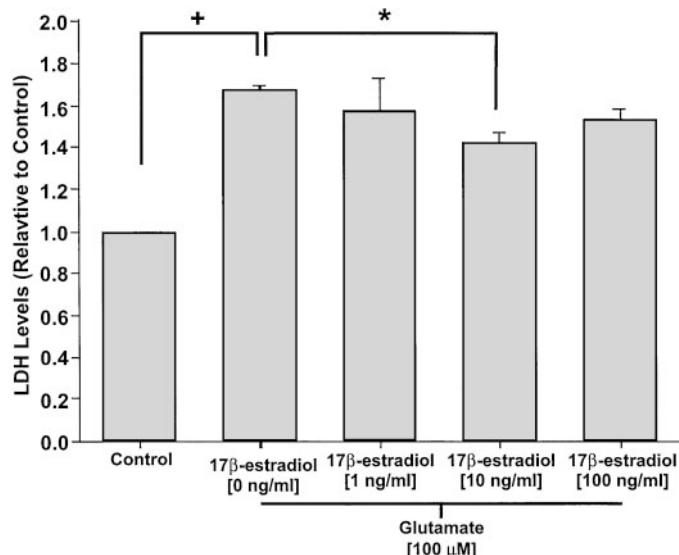


FIG. 1. Effect of 17β -E2 on glutamate toxicity in hippocampal neurons. Primary hippocampal neuron cultures were pretreated with the indicated concentrations of 17β -E2, 48 h before a 5-min exposure to $100\ \mu\text{M}$ glutamate. Twenty-four hours after glutamate exposure, culture medium was assayed for LDH release from damaged cells. +, $P < 0.05$, compared with control cultures; *, $P < 0.05$, compared with vehicle-treated cultures exposed to glutamate alone; $n = 4$ independent experiments, six wells per condition per experiment.

tection we report is effectively similar to that previously reported by other groups using primary cortical cultures, when the data are analyzed in the same manner (15). Maximum neuroprotection, of approximately 18% reduction in LDH release, occurred at $10\ \text{ng/ml}$ 17β -E2 (Fig. 1; $P < 0.05$, compared with glutamate alone; $n = 4$) and was thus used in subsequent experiments.

Effect of progestins on glutamate-induced neurotoxicity

We assessed the effect of three progestins (progesterone, 19-norprogesterone, and MPA) on glutamate-induced neurotoxicity. Progesterone significantly reduced the amount of LDH released into the media 24 h after a 5-min $100\text{-}\mu\text{M}$ glutamate exposure (Fig. 2A). Maximal neuroprotection, of approximately 22% reduction in LDH release, was observed at $10\ \text{ng/ml}$ (Fig. 2A; $P < 0.05$, compared with glutamate alone; $n = 4$). 19-Norprogesterone significantly reduced the amount of LDH released into the media 24 h after a 5-min $100\text{-}\mu\text{M}$ glutamate exposure (Fig. 2B). Maximal neuroprotection, of approximately 21% reduction in LDH release, was observed at $10\ \text{ng/ml}$ (Fig. 2B; $P < 0.05$, compared with glutamate alone; $n = 4$).

In contrast to the other progestins tested, MPA (at either of the concentrations tested) had no effect on LDH release 24 h after a 5-min $100\text{-}\mu\text{M}$ glutamate exposure (Fig. 2C).

Effect of coadministration of 17β -E2 and progestins on glutamate-induced neurotoxicity

Increasingly, progestins are being added to HRT, necessitating the study of the impact of progestin coadministration on the neuroprotective effects of estrogen. A 48-h pretreatment of $10\ \text{ng/ml}$ 17β -E2, before a 5-min $100\text{-}\mu\text{M}$ glutamate

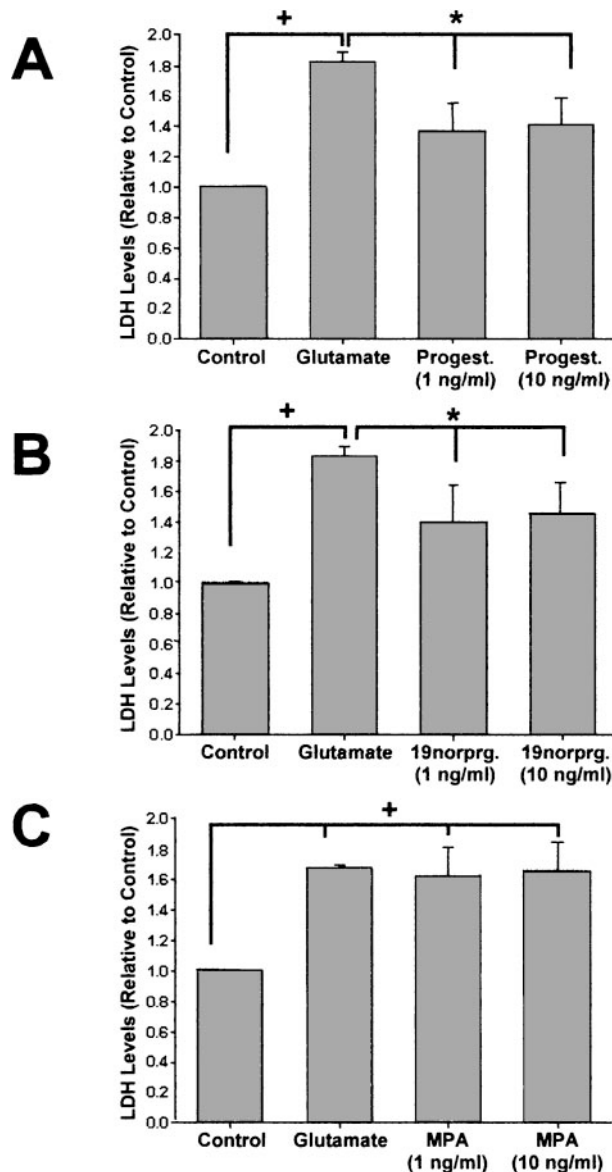


FIG. 2. Effect of progestins on glutamate toxicity in hippocampal neurons. Primary hippocampal neuron cultures were pretreated with the indicated concentrations of progestin, 48 h before a 5-min exposure to $100\ \mu\text{M}$ glutamate. Twenty-four hours after glutamate exposure, the culture media was assayed for LDH release from damaged cells. A, Progesterone (Progesterone); B, 19-norprogesterone (19norprg.); C, MPA. Bars represent mean \pm SEM. +, $P < 0.05$, compared with control cultures; *, $P < 0.05$, compared with vehicle-treated cultures exposed to glutamate alone; $n = 4$ independent experiments, six wells per condition per experiment.

exposure, decreased LDH release approximately 17%, compared with vehicle-treated cultures exposed to glutamate (Fig. 3; $P < 0.05$, compared with glutamate alone; $n = 4$). A slightly larger magnitude of neuroprotection was observed when progesterone (~24% of glutamate alone LDH release) (Fig. 3A; $P < 0.05$, compared with glutamate alone; $n = 4$) or 19-norprogesterone (~23% of glutamate alone LDH release) (Fig. 3B; $P < 0.05$, compared with glutamate alone; $n = 4$) was coadministered along with the E2. In contrast to the other progestins tested, no significant decrease in LDH release

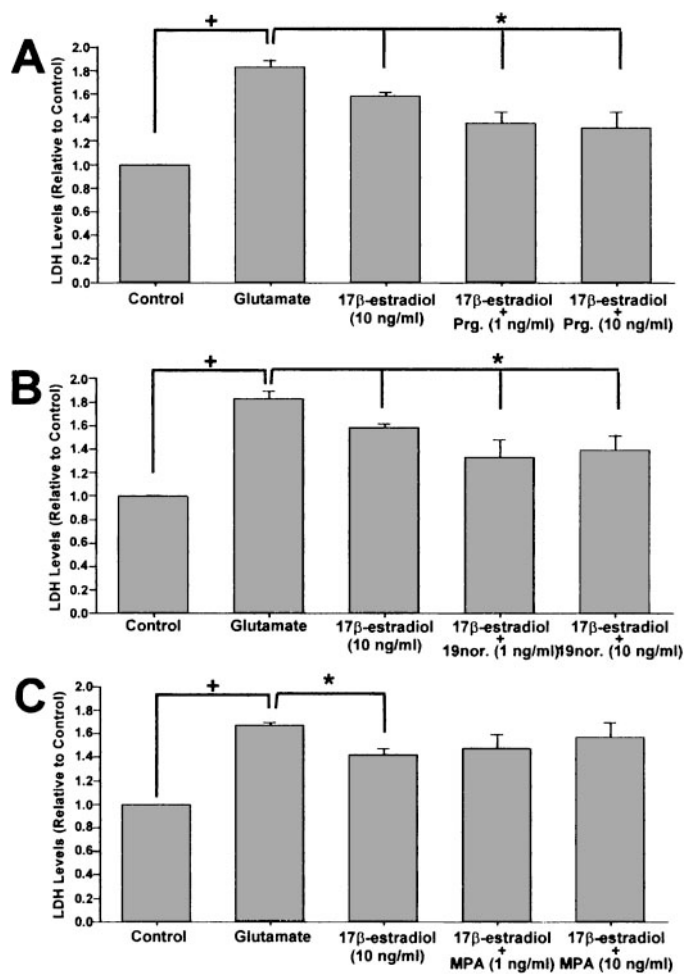


FIG. 3. Effect of 17β -E2 and progestin coadministration on glutamate toxicity in hippocampal neurons. Primary hippocampal neural cultures were pretreated with 17β -E2 (10 ng/ml) with or without the indicated progestin, 48 h before a 5-min exposure to 100 μ M glutamate. Twenty-four hours after glutamate exposure, the culture media was assayed for LDH release from damaged cells. A, Progesterone (Prog); B, 19-norprogesterone (19nor.); C, MPA. Bars represent mean \pm SEM. +, $P < 0.05$, compared with control cultures; *, $P < 0.05$, compared with vehicle-treated cultures exposed to glutamate alone; $n =$ four independent experiments, six wells per condition per experiment.

occurred when MPA was coadministered with 17β -E2, compared with vehicle-treated cultures exposed to glutamate (Fig. 3C; $n = 4$). MPA-induced antagonism of 17β -E2 was greatest at 10 ng/ml (Fig. 3C; $n = 4$). Subsequent experiments used 10 ng/ml of all three progestins.

Effect of 17β -E2 and progestins on MAPK activation

In previous studies, estrogen was shown to activate MAPK with maximal activity at 30 min of treatment (15). Activation of MAPK was shown to be necessary for estrogen-induced neuroprotection (14, 15). To examine the role of MAPK in the modulation of neuroprotection by progestins, MAPK activation in response to hormone treatment was assessed. Antiactive MAPK antibody recognizes ERK MAPK dual phosphorylation on the threonine and tyrosine residues necessary for activation and correlates with ERK1/ERK 2 MAPK ac-

tivation. Aliquots of whole-cell lysates were subjected to SDS-PAGE and probed with antiactive MAPK antibody. Optical density analysis of phospho-ERK2 immunoreactivity was normalized against total ERK2 immunoreactivity.

Treatment with 17β -E2 (10 ng/ml for 30 min) resulted in approximately 60% increase in phospho-ERK2 immunoreactivity (Fig. 4), consistent with previous reports (14, 15). In parallel to effects on neuroprotection, progesterone (10 ng/ml for 30 min) or 19-norprogesterone (10 ng/ml for 30 min) treatment increased phospho-ERK2 immunoreactivity by approximately 123% (Fig. 4A) and approximately 50% (Fig. 4B), respectively. Coadministration of progesterone or 19-norprogesterone with 17β -E2 increased phospho-ERK2 immunoreactivity by approximately 100% (Fig. 4A) and approximately 75% (Fig. 4B), respectively, compared with vehicle-treated cultures. In contrast to its effects on neuroprotection, treatment with MPA (10 ng/ml for 30 min) increased phospho-ERK2 immunoreactivity approximately 60% (Fig. 4C) and did not antagonize E2's effect on MAPK activation when coadministered (Fig. 4C).

Effect of 17β -E2 and progestins on the antiapoptotic protein Bcl-2

An alternate mechanistic pathway leading to neuroprotection is the estrogen-induced increase in the antiapoptotic protein bcl-2 (18–20). To determine whether progestins were regulating the estrogen-inducible Bcl-2 pathway, whole-cell lysates were subjected to SDS-PAGE and probed with anti-Bcl-2 antibody. Densitometric measurement analysis indicated an approximately 2.75-fold higher Bcl-2 immunoreactivity in cultures treated with 17β -E2 (10 ng/ml for 24 h), compared with vehicle-treated control cultures (Fig. 5). Progesterone (10 ng/ml for 24 h) or 19-norprogesterone (10 ng/ml for 24 h) treatment resulted in increases in Bcl-2 expression of approximately 3-fold (Fig. 5A) and approximately 2.6-fold (Fig. 5B), respectively. In contrast, MPA (10 ng/ml for 24 h) did not increase Bcl-2 immunoreactivity, compared with vehicle-treated control cultures (Fig. 5C).

When progesterone or 19-norprogesterone was coadministered with 17β -E2, there was a significant increase in Bcl-2 immunoreactivity of approximately 3-fold (Fig. 5A) and approximately 3.3-fold (Fig. 5C), respectively, compared with vehicle-treated control cultures. Consistent with the results on neuroprotection, coadministration of MPA effectively blocked E2-induced increase in Bcl-2 immunoreactivity (Fig. 5C).

Discussion

The present study demonstrates the divergent actions of different progestins and their impact on estrogen-mediated neuroprotection. Progesterone and 19-norprogesterone, but not MPA, decreased neuronal damage induced by glutamate excitotoxicity. Not only was MPA an ineffective neuroprotectant, but it antagonized estrogen-induced neuroprotection. The treatments affording neuroprotection also resulted in an increase in Bcl-2 expression. Overall, these results suggest that care must be taken in deciding the formulation of HRT, especially with respect to the progestin used.

It is well documented that women have a greater risk than

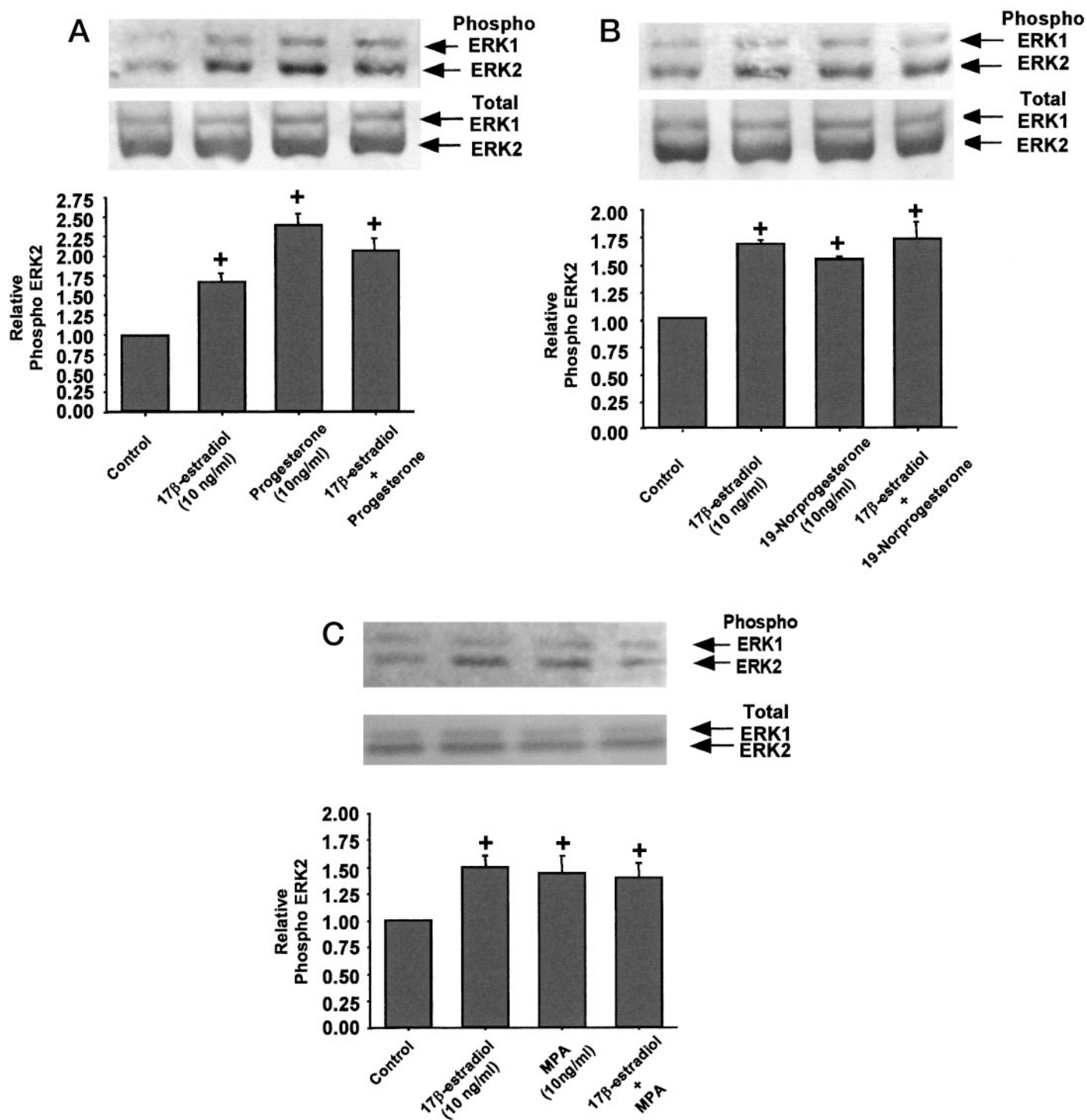


FIG. 4. Activation of MAPK in hippocampal neurons by 17β-E2 and progestin. Primary hippocampal neuron cultures were treated with 17β-E2 (10 ng/ml) with or without the indicated progestins (10 ng/ml) or with vehicle as a negative control for 30 min. Whole-cell lysates were subjected to SDS-PAGE and probed with antiactive MAPK or total ERK antibody. A, Progesterone; B, 19-norprogesterone; C, MPA. Bars represent mean \pm SEM. +, $P < 0.05$, compared with control cultures; $n = 3$ independent experiments.

men to develop AD (26). In addition, females sustain lower mortality and less neuronal damage after cerebral ischemia than males (27). This protection against ischemic brain injury and its related mortality is greatly diminished after menopause or ovariectomy (28). This suggests that the sex-dependent increased risk for neurologic damage is attributable, in

part, to the deprivation of ovarian hormones in postmenopausal women.

Although estrogen has been shown to have neuroprotective effects in numerous experimental models, the clinical data on the protective effects of estrogen replacement therapy (ERT) in women at risk for AD is less clear. Epidemiolo-

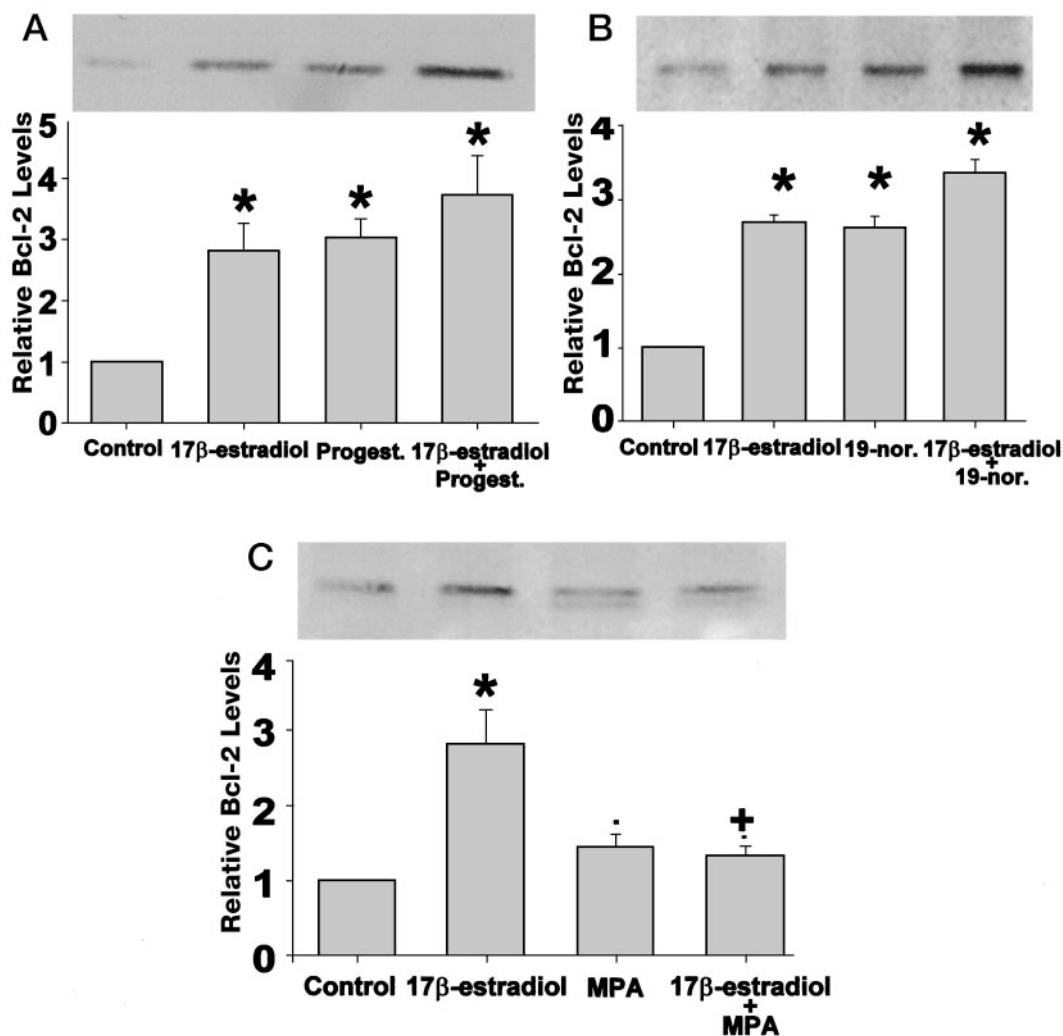


FIG. 5. Effect of 17 β -E2 and progestin on Bcl-2 expression in hippocampal neurons. Primary hippocampal neuron cultures were treated with 17 β -E2 (10 ng/ml) with or without the indicated progestins (10 ng/ml) or with vehicle as a negative control for 24 h. Whole-cell lysates were subjected to SDS-PAGE and probed with anti-Bcl-2 antibody. A, Progesterone; B, 19-norprogesterone; C, MPA. Bars represent mean \pm SEM. +, $P < 0.05$, compared with control cultures; *, $P < 0.05$, compared with 17 β -E2-treated cultures; $n = 3$ independent experiments.

logical studies have demonstrated that women who receive ERT are less likely to develop AD or develop AD with a later onset (27, 29–31). However, some studies have reported no effect of hormones on the development of AD (32).

Vongher and Frye (22) found that rats treated with progesterone, alone or in conjunction with estrogen, before neural insults, had improved cognitive behavior. Other groups have reported no effect of progestin on neuroprotection (21). Here, we show that progesterone, as well as 19-norprogesterone, protects hippocampal neurons against glutamate excitotoxicity. This effect is seen when progesterone is administered alone or in conjunction with 17 β -E2. In contrast, MPA is ineffective when administered alone. When administered in conjunction with E2, it antagonizes the neuroprotective effect of E2. This fits with the clinical data indicating negative effects of synthetic progestins in the treatment of women with AD. Two recent clinical studies found beneficial effects of long-term ERT on cognitive function, but these improvements were abrogated by the addition of MPA (33, 34).

Although ER and PR generally function as transcription

factors, a large body of evidence now documents rapid effects of estrogen that are not in accordance with classical genomic mechanisms of hormone action (13, 35). In particular, attention has been focused on the ability of estrogen to activate MAPK pathways, including p42/44 (ERK1/ERK2) (12–14). Furthermore, estrogen-mediated neuroprotection is thought to involve MAPK activation, because inhibitors of tyrosine kinase and MAPK blocked the neuroprotective effect of estrogen against glutamate toxicity (14, 15, 36). Our results show that neuroprotective concentrations of 17 β -E2, progesterone, and 19-norprogesterone cause rapid activation of MAPK. Surprisingly, MPA, which blocked estrogen-induced neuroprotection, also activated MAPK when administered alone or in conjunction with 17 β -E2. Although previous studies showed that blocking MAPK activation inhibited estrogen-induced neuroprotection, indicating a necessity for MAPK activation, our results imply that MAPK activation is necessary, but not sufficient, for protection. Alternatively, different upstream activators may produce different MAPK-induced effects, as shown for fibroblast growth

factor, NGF, and epithelial growth factor effects on PC12 cells. Fibroblast growth factor and NGF induce differentiation, whereas epithelial growth factor acts as a mitogen, even though all three activate MAPK (37). It is possible that a similar mechanism accounts for the different effects of progestins that all activate MAPK.

An alternative proposed mechanism of estrogen-mediated neuroprotection is up-regulation of Bcl-2 expression. The protein Bcl-2, initially identified in hematopoietic tissues, has more recently been found to be ubiquitous. Its main function seems to be to override apoptosis. Bcl-2 has been shown to inhibit neuronal death caused by multiple insults, including growth factor withdrawal, free radicals, and glutamate excitotoxicity (17, 38, 39). Estrogen has been shown to increase Bcl-2 expression in many tissues types, including primary neuronal cultures and neural cell lines (18–20). Here, we confirm that there is an E2-induced increase in Bcl-2 expression in primary neurons. Progesterone or 19-norprogesterone, administered alone or in conjunction with 17 β -E2, resulted in increased Bcl-2 expression as well. Consistent with MPA inhibition or blockade E2-induced neuroprotection, MPA blocked the estrogen-induced increase in Bcl-2 expression. This further implicates the role of Bcl-2 in ovarian hormone-mediated neuroprotection.

A previous study on breast cancer cells showed a negative regulatory effect of progestin on Bcl-2 expression (40). Although the negative regulatory effect may reflect a difference in response between breast cancer cells and neurons, the progestin used was the synthetic pregnane progestin, OR2058. This synthetic progestin may act more like the synthetic progestin MPA than like natural progesterone. This stresses further the need to study structurally different progestins for differing effects.

Considered together, these results are consistent with the emerging clinical data indicating divergent actions of different progestins. Studies comparing the associations between unopposed estrogen use and combined estrogen-progestin therapy, including the Postmenopausal Estrogen/Progestin Interventions trial, have shown that MPA, but not micronized progesterone, blunted the beneficial association between estrogen and high-density lipoprotein cholesterol (41, 42). Likewise, even though progesterone can protect against increased risk of breast cancer associated with unopposed estrogen (6), the risk of breast cancer was higher with MPA plus estrogen than with estrogen alone (7). The Kame project reported that the beneficial association between estrogen and cognitive change was opposed by the addition of MPA (34). Here, we show that progesterone and 19-norprogesterone plus E2 protected, but MPA plus E2 failed to protect, against neurotoxic insults. These findings may have important implications for the effective use of HRT in the maintenance of neuronal function during menopause and aging and for protection against neurodegenerative diseases such as AD.

Acknowledgments

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