Brief Communications

Estradiol-Induced Increase in the Magnitude of Long-Term Potentiation Is Prevented by Blocking NR2B-Containing Receptors

Caroline C. Smith and Lori L. McMahon
Department of Physiology and Biophysics, University of Alabama at Birmingham, Birmingham, Alabama 35294

Estradiol, through activation of genomic estrogen receptors, induces changes in synaptic morphology and function in hippocampus, a brain region important for memory acquisition. Specifically, this hormone increases CA1 pyramidal cell dendritic spine density, NMDA receptor (NMDAR)-mediated transmission, and the magnitude of long-term potentiation (LTP) at CA3–CA1 synapses. We recently reported that the estradiol-induced increase in LTP magnitude occurs only when there is a simultaneous increase in the fractional contribution of NMDAR-mediated transmission relative to AMPA receptor transmission, suggesting a direct role for the increase in NMDAR transmission to the heightened LTP magnitude. Estradiol has been shown to increase expression of the NMDAR subunit NR2B, but whether this translates into an increase in function of NR2B-containing receptors remains to be determined. Here we show that not only is the estradiol-induced increase in NMDAR transmission mediated by NR2B-containing receptors, but blocking these receptors using RO25-6981 \( [R-(R,S)-\alpha-(4-hydroxyphenyl)-\beta-methyl-4-(phenethyl)-1-piperidine propanol] \) (0.5 \( \mu M \)), an NR2B selective antagonist, prevents the estradiol-induced increase in LTP magnitude. Thus, our data show a causal link between the estradiol-induced increase in transmission mediated by NR2B-containing NMDARs and the increase in LTP magnitude.

Key words: estradiol; NMDA receptor; synaptic plasticity; hippocampus; LTP; NR2B

Introduction

Elevated estradiol levels increase memory acquisition, and, when hormone levels decline in aging, memory is impaired (Phillips and Sherwin, 1992a,b; Rapp et al., 2003; Daniel et al., 2006). However, the mechanisms mediating this change in memory are unclear. In hippocampus, estradiol increases CA1 pyramidal cell dendritic spine density, NMDA receptor (NMDAR) transmission, and the magnitude of long-term potentiation (LTP) (Gould et al., 1990; Woolley et al., 1996; Warren et al., 1995; Cordoba Montoya and Carrer, 1997; Bi et al., 2001; Maren, 2001; Hao et al., 2003; Smith and McMahon, 2005), all of which require activation of genomic estrogen receptors (ERs) (Smith and McMahon, 2005). Because changes in spine density and synaptic efficacy in area CA1 of hippocampus are associated with learning and memory mechanisms (Tsien et al., 1996; Leuner and Shors, 2004; Malenka and Bear, 2004), the estradiol-mediated alterations in synapse density and function may be causally linked to the hormone-induced improvements in memory acquisition.

Previous studies have suggested that the estradiol-induced increase in NMDAR transmission underlies the increase in LTP magnitude (Foy, 2001; Smith and McMahon, 2005). Recently, we reported that the increase in LTP magnitude with estradiol treatment occurs only when transmission mediated by NMDARs is increased relative to AMPA receptor (AMPAR)-mediated transmission (Smith and McMahon, 2005). Thus, our findings are consistent with a critical role for the increase in NMDAR transmission to the increase in LTP magnitude (Smith and McMahon, 2005).

Induction of LTP has been shown to require activation of NR2A-containing NMDARs, whereas LTD induction requires activation of NMDARs containing NR2B subunits (Liu et al., 2004). Conversely, overexpressing NR2B subunits increases the magnitude of LTP (Tang et al., 1999; Barria and Malinow, 2005), and this increased plasticity is reversed when NR2B subunits are replaced by NR2A subunits (Barria and Malinow, 2005). Thus, an increase in function of NMDARs containing either NR2A or NR2B subunits can facilitate an increase in LTP magnitude.

Estradiol increases NR2B subunit mRNA, the number of NR2B binding sites, and the synaptic localization of NR2B-containing receptors (Cyr et al., 2001; Adams et al., 2004). It is currently unknown whether the estradiol-induced increase in NMDAR transmission is attributable to the increase in synaptic localization of NR2B-containing receptors. Furthermore, whether the increase in synaptic NR2B-containing receptors results from expression of new receptors or from lateral movement of existing receptors into the synapse is an open question (Cyr et al., 2001; Adams et al., 2004).

The goal of this study was to investigate whether NR2B-
containing NMDARs participate in the estradiol-induced increase in NMDAR transmission and LTP magnitude. We find that the increase in amplitude of evoked NMDAR EPSCs recorded in slices from estradiol-treated animals is attributable to an increase in current mediated by NR2B-containing receptors. Moreover, blocking these receptors prevents the increase in LTP magnitude. Thus, our data provide a causal link between an increase in current mediated by NR2B-containing receptors and the increase in LTP magnitude.

Materials and Methods

Animal treatment
Experimental procedures were approved by the University of Alabama Institutional Animal Care and Use Committee and followed the National Institutes of Health experimental guidelines.

Surgical procedures and hormone treatments were performed as described previously (Smith and McMahon, 2005). Briefly, 7- to 9-week-old female Sprague Dawley rats were ovarioctomized, injected twice (24 h interval) subcutaneously with either 10 μg of 17β-estradiol (E) in 100 μl of cotton seed oil or oil alone (Woolley and McEwen, 1993), and divided into the following groups: oil vehicle-treated control (V), E24, E48, E72, and E120 (number corresponds to hours after the second estradiol injection). The dry uterine weight was examined to confirm completion of estrus (Hall et al., 1992) (uterine weight: vehicle, 0.10 ± 0.01 g; E24, 0.24 ± 0.01 g; E48, 0.21 ± 0.02 g; E72, 0.17 ± 0.01 g; E120 0.17 ± 0.01 g).

Hippocampal slice preparation
For patch-clamp recordings, brains were removed, and coronal slices (400 μm) were cut from dorsal hippocampus in a high-sucrose, low-Na⁺ artifical CSF (aCSF) [in mM: 85 NaCl, 2.5 KCl, 4 MgSO₄, 0.5 CaCl₂, 1.25 NaH₂PO₄, 25 NaHCO₃, 25 glucose, 75 sucrose, 2 kynurenic acid, and 0.5 ascorbate (saturated with 95% O₂ and 5% CO₂, pH 7.4)] and were held for 30 min before being transferred to a standard aCSF (below) containing 2 mM kynurenic acid. For extracellular recordings, slices were cut and stored in standard aCSF (in mM): 119 NaCl, 26 NaHCO₃, 2.5 KCl, 1 NaH₂PO₄, 2.5 CaCl₂, 1.3 MgSO₄, and 10 glucose (saturated with 95% O₂ and 5% CO₂, pH 7.4). Slices were maintained for up to 4 h in a submersión chamber at room temperature continuously bubbled with 95% O₂ and 5% CO₂.

Electrophysiology
All recordings were performed in a submersión chamber perfused with aCSF (26–28°C) at 2–3 ml/min and continuously bubbled with 95% O₂ and 0.5 ascorbate (saturated with 95% O₂ and 5% CO₂).

Electrophysiological recordings were obtained from somas of CA1 pyramidal cells using the “blind” patch technique (input resistance, 70–160 MΩ; series resistance, 15–25 MΩ). Electrodes (3–6 MΩ) were filled with the following (in mM): 117 cesium glutamate, 0.6 EGTA, 2.8 NaCl, 5 MgCl₂, 2 ATP, 0.3 GTP, 20 HEPES, and 5 QX-314 N-(2,6-Dimethylphenylcarbamoylmethyl) triethylammonium bromide. A stimulating electrode was placed in stratum radiatum within 100–200 μm of the recorded cell, and the stimulus intensity (0.1 Hz, 100 μs duration) was set to elicit evoked glutamatergic EPSCs of 100–200 pA. To measure NMDAR currents, cells were held at −20 mV to relieve the voltage-dependent Mg⁺ block. NMDAR currents were isolated by blocking AMPAR and GABA_α receptors with 10 μM DNQX and 100 μM picrotoxin, respectively. The CA3 region was removed to minimize epileptic activity. To ensure that the remaining current was mediated by NMDARs, the NMDAR antagonist D,L-APV (100 μM) was bath applied at the end of the each experiment (see Fig. 1E). AMPAR currents were obtained by either subtracting the pharmacologically isolated NMDAR currents from the glutamatergic EPSCs or pharmacologically isolating AMPAR currents and then obtaining the NMDAR current by subtraction. When indicated, NR2B-containing receptor currents were blocked by bath applying 0.5 μM RO25-6981 [R-(R,S)-α-(4-hydroxyphenyl)-β-methyl-4-(phenylmethyl)-1-piperidine propanol] (RO) (NR2B-selective antagonist). The current mediated by NR2B-containing receptors was calculated by subtracting the RO25-6981-insensitive current from NMDAR-mediated EPSCs. If either input or series resistance varied by more than 10%, the experiment was excluded. Signals were collected using an Axopatch-1D amplifier (Molecular Devices, Sunnyvale, CA) in voltage-clamp mode, at 5× gain, filtered at 2 kHz, and acquired in software written in Labview (gift from Dr. Richard Mooney, Duke University, Durham, NC). Statistical significance was determined using either Student’s t test or one-way ANOVA with Tukey’s post hoc analysis when indicated.

LTP experiments. LTP experiments were performed as described previously (Smith and McMahon, 2005). Briefly, a 20 min baseline was acquired by stimulating (0.1 Hz, 100 μs duration) within 400 μm of the extracellular recording electrode (filled with 2 mM NaCl) in area CA1 to yield field EPSP (fEPSPs) of 0.6–0.7 mV in amplitude (~50% of maximum). Experiments were excluded if there was more than 8% variance in baseline. LTP was induced using the following (Smith and McMahon, 2005): 100 Hz tetanus, 0.5 s duration at 1.5× baseline stimulus intensity, applied four times, 20 s intervals. LTP experiments with or without RO25-6981 were interleaved in slices from vehicle and E24 animals. Signals were collected using an Axoclamp-2A amplifier (Molecular Devices) in bridge mode, at 1000× gain, filtered, and acquired as above. Statistical significance was determined using one-way ANOVA and Tukey’s post hoc analysis.

Results

The estradiol-induced increase in NMDAR EPSC amplitude is mediated by NR2B-containing receptors
Similar to our recent results using extracellular dendritic field potential recordings (Smith and McMahon, 2005), we find in whole-cell voltage-clamp recordings a significant increase in amplitude of evoked NMDAR currents at E24 (268 ± 17% increase) and E48 (266 ± 30% increase) with no statistical difference between these two time points (E24 or E48 vs V, p < 0.001; E24 vs E48, p > 0.05; E72 or E120 vs V, p > 0.05; unpaired Student’s t test) (Fig. 1A, B). We next wanted to determine whether the increase in NMDAR amplitude is attributable to an increase in current mediated by NR2B-containing receptors. We find a significant contribution of current mediated by NR2B-containing receptors to the NMDAR-evoked EPSC amplitude in cells from slices in all experimental groups (E24 vs E24 + RO, p < 0.001; E48 vs E48 + RO, p < 0.001; V vs V + RO, p < 0.001; E72 vs E72 + RO, p < 0.01; E120 vs E120 + RO, p < 0.01; paired Student’s t test) (Fig. 1C). Importantly, at E24 and E48, there is an approximate fivefold increase in current mediated by NR2B-containing receptors compared with vehicle (E24, 559 ± 65%; E48, 567 ± 79%; E24 or E48 vs V, p < 0.001; E24 vs E48, p > 0.05; unpaired Student’s t test) (Fig. 1C). Furthermore, the fractional contribution of the NR2B-mediated current to the evoked NMDAR EPSC amplitude is significantly increased at E24 and E48 compared with vehicle (E24 or E48 vs vehicle, p < 0.001) (Fig. 1B). In addition, the mean amplitude of the RO25-6981-insensitive current is not significantly different between any of the experimental groups (p > 0.05, ANOVA) (Fig. 1A, B). Thus, we find that the increase in NMDAR-mediated EPSC amplitude is a direct result of an increase in current mediated by NR2B-containing receptors.

The estradiol-induced increase in the NMDAR/AMPAR ratio at E24 is prevented by blocking NR2B-containing receptors
We next wondered whether the estradiol-induced increase in function of NR2B-containing receptors is responsible for the increase in the fractional contribution of NMDARs to AMPARs observed in extracellular dendritic field potential recordings (Smith and McMahon, 2005). We investigated this by further analyzing the glutamate current from cells shown in Figure 1. The NMDAR/AMPAR ratio was calculated for each cell by dividing...
NR2B-containing receptors are responsible for the estradiol-induced increase in NMDAR current. A. Waveforms of NMDAR (open boxes) and RO25-6981-insensitive EPSCs (hatched boxes). B. Bar chart of NMDAR EPSCs (open bars), RO25-6981-insensitive EPSCs (hatched bars), and EPSCs mediated by NR2B-containing receptors (solid bars, obtained by subtraction) in cells from vehicle (V), E24, E48, E72, and E120. The NMDAR EPSC amplitude (open bars) is increased at E24 (117 ± 48 pA; n = 9 cells/8 animals) and E48 (168 ± 1 pA; n = 9 cells/8 animals) compared with vehicle (64 ± 4 pA; n = 9 cells/8 animals) (E24 or E48 vs V, p < 0.001) but not at E72 or E120 (E72, 44 ± 5 pA; n = 11 cells/8 animals; E120, 49 ± 4 pA; n = 12 cells/9 animals) (E24 or E48 vs V, p < 0.001). NR2B-mediated current amplitude (filled bars) is increased at E24 (68 ± 8 pA; n = 8 cells/7 animals) and E48 (69 ± 10 pA; n = 9 cells/8 animals) compared with vehicle (12 ± 2 pA; n = 9 cells/8 animals; E24 or E48 vs V, p < 0.001) but not at E72 or E120 (E72, 15 ± 3 pA; n = 7 cells/7 animals, p > 0.05; E120, 20 ± 3 pA; n = 11 cells/8 animals; E72 or E120 vs V, p > 0.05). RO25-6981-insensitive currents (hatched bars) are not different between groups (vehicle, 32 ± 4 pA; n = 9 cells/8 animals; E24, 47 ± 6 pA; n = 8 cells/7 animals; E48, 47 ± 7 pA; n = 9 cells/8 animals; E72, 27 ± 3 pA; n = 7 cells/7 animals; E120, 29 ± 4 pA; n = 11 cells/8 animals; p > 0.05, ANOVA and Tukey’s post hoc test). C. Plot of a representative experiment from a cell recorded in a vehicle-treated slice. Symbols indicate where waveforms were obtained. Statistical significance was determined using unpaired Student’s t test except as noted. In all figures, waveforms shown are averages of 10 events. Error bars represent SEM.

The estradiol-induced increase in LTP magnitude at E24 is prevented when NR2B-containing receptors are blocked. Using dendritic field potential recordings, we directly tested whether the increase in transmission mediated by NR2B-containing receptors is required for the increase in LTP magnitude induced with high-frequency stimulation (HFS). We chose to assess the effect of RO25-6981 on the magnitude of LTP at E24 because the estradiol-induced increase in LTP is greatest at this time point (Smith and McMahon, 2005). There were no statistical differences in baseline EPSP slope between groups; therefore, data were normalized for ease of comparison (V, 0.20 ± 0.01 mV/ms; E24, 0.20 ± 0.01 mV/ms; V + RO, 0.20 ± 0.01 mV/ms; E24 + RO, 0.19 ± 0.06 mV/ms; p > 0.05). We find that RO25-6981 has no effect on the magnitude of LTP in slices from vehicle-treated control animals, but it completely blocks the estradiol-induced increase in LTP magnitude (E24 vs V, p < 0.004; E24 + RO vs V, p > 0.05; V + RO vs V, p > 0.05; one-way ANOVA, Tukey’s post hoc test) (Fig. 3), indicating a causal relationship between the increase in transmission mediated by NR2B-containing receptors and the increase in LTP magnitude. Next, we investigated whether the increase in steady-state depolarization during HFS observed previously (Smith and McMahon, 2005) is attributable to NR2B-containing receptors and find that, although there is not a statistically significant effect, there is a trend toward less depolarization in the presence of RO25-6981 (E24 vs V, p < 0.05; E24 vs E24 + RO, p > 0.05). This finding demonstrates that NR2B-containing receptors may play a role in the increase in steady-state depolarization, but clearly other mechanisms must be required for this increase to occur.
Discussion

The major finding of this study is that the estradiol-induced increase in LTP magnitude requires activation of NR2B-containing receptors. This role of NR2B subunits is supported by recent reports demonstrating that overexpressing NR2B subunits (in transgenic animals and virally transfected hippocampal slices) increases LTP magnitude (Tang et al., 1999; Barria and Malinow, 2005). Our findings extend the idea of a causal relationship between an increase in NR2B-containing receptors and LTP magnitude by demonstrating that a physiological stimulus in vivo (i.e., elevated estradiol levels) can increase plasticity by increasing the function of NR2B-containing receptors. This increase in postsynaptic current we observe could be attributable to an increase in NR2B subunit expression or phosphorylation. Because presynaptic NR2B-containing receptors have been found in other brain regions, we cannot rule out a potential contribution of extrasynaptic NR2B-containing receptors to this increase in LTP magnitude induced after estradiol treatment (Zamani et al., 2004).

Does estradiol increase the expression of NR2B subunits in new spines? We think this is likely because previous studies have demonstrated that before postnatal day 14, NR2B expression and silent synapse density are increased (Petralia et al., 1999; Sans et al., 2000; Law et al., 2003). We report that estradiol increases NR2B-containing receptor-mediated currents during the same time frame that we have shown this hormone increases the density of dendritic spines (Smith and McMahon, 2005). Therefore, it is possible that these NR2B-containing receptors are in the new spines and thus are silent synapses. As synapses mature during development, synaptically located NR2B-containing receptors are replaced by receptors containing NR2A subunits coincident with an increase in currents mediated by AMPARs (Law et al., 2003; Owen et al., 2004). This is similar to what we previously have shown occurs at E72, when the fractional contribution of transmission mediated by NMDARs and AMPARs is restored to that in slices from vehicle-treated animals, although spine density and overall transmission remain elevated (Smith and McMahon, 2005). Perhaps, with time after hormone treatment, the newly formed, potentially silent synapses undergo a maturation into active synapses, expressing both NMDARs and AMPARs. This change may begin as early as E48 because we observe an increase in AMPAR transmission at this time point (Smith and McMahon, 2005). However, the NMDAR/AMPAR ratio at E48, similar to E24, is still significantly different from vehicle, but, by E72, this ratio is reset to that measured in slices from vehicle-treated control slices. Thus, estradiol could exploit mechanisms used during development to temporarily induce synapses with immature characteristics into an otherwise mature hippocampal circuit after hormone treatment and during the normal estrous cycle by upregulating transmission mediated by NR2B-containing receptors in silent synapses.

We have shown previously that tamoxifen blocks the estradiol-induced increase in LTP magnitude, spine density, and NMDAR transmission (Smith and McMahon, 2005), indicating a requirement for genomic estrogen receptor activation. However, whether these changes are attributable to ERα and ERβ, both of which can be found in hippocampus (McEwen et al., 2001), has not been determined. ERs are localized to cholinergic terminals and GABAergic interneurons (Hart et al., 2001; Towart et al., 2003), and it is believed that an increase in acetylcholine release and a decrease in GABAergic transmission triggers the increase in spine density and NMDAR expression (Rudick and Woolley, 2001; Gabor et al., 2003). Thus, ERs is likely responsible for the changes in hippocampal morphology and function with estradiol treatment.

Estradiol increases NMDAR and AMPAR phosphorylation, decreases the afterhyperpolarization, and increases Ca2+ currents mediated by voltage-gated Ca2+ channels (Wong and Moss, 1992; Foy et al., 1999; Pozzo-Miller et al., 1999; Kurata et al., 2001; Kumar and Foster, 2002; Scharfman et al., 2003), any or all of which could underlie the increase in LTP magnitude. Additionally, estradiol increases BDNF expression (Solum and Hanada, 2002), and, similar to estradiol, BDNF increases hippocampal excitability, NMDAR transmission, LTP magnitude, and spine density (Murphy et al., 1998; Crozier et al., 1999; Scharfman et al., 2003; Kramer et al., 2004). Furthermore, estradiol decreases GABA responses by activation of protein kinase Cα and protein kinase A, which could contribute to the increase in LTP magnitude (Qiu et al., 2003). Thus, to fully understand...
how estradiol induces changes in hippocampus, it is important to know the mediators downstream of estrogen receptor activation that are critical for the increase in spine density, NMDAR transmission, and LTP magnitude.

This is the first study to show a causal link between estradiol and the increase in LTP magnitude. Additional work is required to more fully define the cellular mechanisms mediating these increases in these receptors in newly formed or existing synapses. A clear understanding of these processes could provide insight into the mechanisms mediating the hormone-induced improvements in learning and memory.

**References**


Barria A, Malinow R (2005) NMDA receptor subunit composition controls synaptic plasticity by regulating binding to CaMKII. Neuron 48:289–301.


Smith CC, McMahon LL (2005) Estrogen-induced increase in the magnitude of long-term potentiation occurs only when the ratio of NMDA transmission to AMPA transmission is increased. J Neurosci 25:7780–7791.


