17β-Estradiol Enhances NMDA Receptor-Mediated EPSPs and Long-Term Potentiation


INTRODUCTION

Estrogenic steroids are reported to alter electrophysiological, biochemical, and morphological properties of mammalian CNS neurons and glial cells (Brinton 1993; Brinton et al. 1997a,b; Gould et al. 1990; Murphy and Segal 1996; Simerly et al. 1990; Stone et al. 1998; Wong and Moss 1991; Woolley et al. 1990). Although modification of gene expression as a consequence of estrogen liganding to DNA-binding receptors is the traditional framework for interpreting underlying mechanisms, an increasing number of reports document effects of acute application of estrogenic steroids that are too rapid (occurring within ≈10 min) to be accounted for by a genomic pathway. In particular, estrogenic steroid-induced changes in neuronal excitability suggest other, non-genomic mechanisms involving a direct interaction with sites of the plasma membrane to regulate ligand-gated ion channels and neurotransmitter transporters (Wong et al. 1996). Effects of estrogen on the electrophysiological activity of rodent hippocampal neurons were first reported by Teyler et al., who found that 17β-estradiol treatment induced a rapid (≈10 min) enhancement of glutamatergic synaptic transmission in the CA1 region of in vitro hippocampal slices (Teyler et al. 1980). Subsequent reports indicated that only the biologically active isomer of estrogen, 17β-estradiol, and not the 17α-estradiol isomer is effective in eliciting these short-term electrophysiological effects (Foy and Teyler 1983; Wong and Moss 1991, 1992). On the basis of intracellular in vitro recordings from CA1 pyramidal cells, Wong and Moss (1992) reported that administration of 17β-estradiol increased synaptic excitability by enhancing the magnitude of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-mediated responses. The rapid onset of increased excitability and its blockade by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, an AMPA receptor antagonist) and not D-2-amino-5-phosphonovalerate [D-APV, a competitive N-methyl-D-aspartate (NMDA) receptor antagonist] supported a postsynaptic membrane site of action and an expression by non-NMDA receptor-channels. Later studies with whole cell recordings found that acute 17β-estradiol application potentiated kainate-induced currents in a subpopulation (38%) of CA1 cells (Gu and Moss 1996), although a direct interaction between 17β-estradiol and the receptor channel was not indicated (Wong and Moss 1994). In total, the work of Moss and colleagues supports the possibility of a second messenger mechanism underlying the rapid effects of 17β-estradiol, most likely involving a G-protein coupled AMPA-dependent phosphorylation event.

The apparently exclusive estrogenic steroid modification of non-NMDA receptor channels stands in contrast to a large body of evidence demonstrating 17β-estradiol regulation of NMDA receptor-mediated function. Morphological studies on the course of neuronal development conducted in vitro in our laboratory (Brinton et al. 1997a,b) have shown that estrogenic steroids exert a growth-promoting, neurotrophic effect on hippocampal and cortical neurons via a mechanism that requires activation of NMDA receptors. Moreover, the neurotrophic effects of estrogenic steroids can be blocked by an NMDA receptor antagonist in cultured neurons before synaptic contacts occur (Brinton et al. 1997b). In vivo studies by Woolley and McEwen (1994) revealed a proliferation of dendritic spines...
after 17β-estradiol treatment that can be prevented by blockade of NMDA receptor channels, although not by AMPA or muscarinic receptor antagonists. Other reports provide evidence that chronic 17β-estradiol treatment increases the number of NMDA receptor binding sites and NMDA receptor-mediated responses (Gazzaley et al. 1996; Woolley et al. 1997).

The possibility of direct regulation of NMDA receptor-mediated synaptic transmission by 17β-estradiol may not have been detected previously because tests of this hypothesis are so few in number and, more importantly, have yet to be conducted under optimal conditions. Because of the voltage-dependent blockade of the NMDA channel by Mg$^{2+}$ and the slow kinetics of ligand-gated channel opening relative to that of the AMPA receptor subtype, there is only a minor NMDA receptor-mediated component of the excitatory postsynaptic potential (EPSP) evoked by low-frequency stimulation of glutamatergic afferents. This NMDA component can be enhanced with low Mg$^{2+}$ concentration or high-frequency stimulation to induce the depolarization accompanying the summation of overlapping EPSPs (Xie et al. 1992). In the experiments reported here, we used both the conditions of low Mg$^{2+}$ and high-frequency stimulation in separate experiments to examine the acute effects of 17β-estradiol on pharmacologically isolated NMDA receptor-mediated EPSPs in CA1 pyramidal cells to determine if estradiol alters NMDA receptor-channel activity. In other experiments, we investigated the acute effects of 17β-estradiol treatment on the induction and expression of long-term potentiation (LTP), an enduring enhancement of glutamatergic synaptic transmission that, in the hippocampal CA1 region, requires high-frequency stimulation sufficient to activate NMDA receptor-channels.

**METHODS**

Transverse hippocampal slices (400 μm) were prepared from experimentally naive, 200- to 350-g adult male Sprague-Dawley rats (Harlan). Slices were incubated in an artificial cerebrospinal fluid (aCSF) perfusion medium that consisted of (in mM) 126 NaCl, 5 KCl, 1.25 NaH$_2$PO$_4$, 26 NaHCO$_3$, 10 glucose, 2 CaCl$_2$, 2 MgCl$_2$, 2 ascorbic acid, bubbled with 95% O$_2$-5% CO$_2$ and maintained at 32 ± 0.5°C. For intracellular recordings, slices were superfused (~3 ml/min) in a submerged chamber with aCSF containing bicuculline (5 μM) and either the AMPA receptor antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX) (10 μM) and a reduced Mg$^{2+}$ concentration (0.1 mM) to isolate NMDA receptor-mediated EPSPs or the NMDA receptor antagonist D-2-amino-5-phosphonovalerate (D-APV; 50 μM) and a higher Mg$^{2+}$ concentration (1 mM) to isolate AMPA receptor-mediated EPSPs. CA1 pyramidal cell EPSPs were recorded intracellularly with glass microelectrodes filled with 2 M potassium acetate (resistance: 90 –150 MΩ). Depolarizing and hyperpolarizing current injections were applied for measurement of input resistance to generate voltage steps across the neuronal membrane for calculation of input resistance. Experiments were conducted only when stable intracellular recordings were obtained from neurons with a resting membrane potential of -60 to -70 mV.

**FIG. 1.** The amplitude of N-methyl-d-aspartate (NMDA) receptor-mediated excitatory postsynaptic potentials (EPSPs) in CA1 pyramidal cells is increased shortly after the addition of 1 nM 17β-estradiol to the perfusion medium. **A**. top trace: EPSPs evoked when slice was perfused with medium including 1.0 mM Mg$^{2+}$ and in the absence of the non-NMDA receptor antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX). **Bottom trace**: NMDA receptor-mediated EPSPs evoked 10 min after medium was switched to include 0.1 mM Mg$^{2+}$ and 10 μM of the non-NMDA receptor antagonist DNQX. **B**: 1 nM 17β-estradiol potentiated the isolated EPSPs within 3 min. A, B, and C show EPSP amplitude measurements from 9 to 12 cells. **C**: average amplitude of NMDA-mediated EPSPs after 17β-estradiol perfusion was increased in both EPSPs evoked by paired pulse stimulation. EPSPs from control slices were normalized, and potentiated EPSP amplitudes were extracted from recorded data. * P < 0.05 (n = 9).
estradiol on LTP was studied with high-frequency stimulation (after
isolation of non-NMDA receptor-mediated EPSPs. A
eral stimulation and recorded at 0.1 Hz, with 17
brane potential of at least
be perfused with aCSF or aCSF containing 17
after a stable, 10-min baseline period. Field EPSP responses of the two groups were
ated in duration, with slow rise and fall times characteristic of NMDA receptor-mediated synaptic responses (Xie et al. 1992). Identification of DNQX-resistant responses as being NMDA receptor mediated was confirmed by the effects of D-APV, which completely abolished residual evoked synaptic activity (Fig. 1).

At 1 nM, 17β-estradiol induced a rapid increase in the amplitude of the NMDA receptor-mediated EPSPs evoked by paired impulse stimulation of Schaffer collateral input in 9 of 13 cells. Measured at ~6 min after application of 17β-estradiol, the enhancement occurred for both of the paired responses (Fig. 1C). The mean increase of the first response was 180 ± 26%, P < 0.05, and that of the second response was 197 ± 22%, P < 0.01. After correcting for the lag time of the perfusion system (~1.5 min), the latency for onset of the 17β-estradiol effect was <2 min for all cells. In some cells, the enhanced EPSPs reached the firing threshold after a longer (>10 min) 17β-estradiol application period (Fig. 1B). For two cells, the enhanced EPSPs were further confirmed as NMDA receptor mediated by their nearly complete blockade after bath application of 50 μM D-APV (Fig. 1B).

In another series of experiments, the AMPA receptor was pharmacologically isolated by applying the NMDA receptor antagonist D-APV (50 μM) to the bath. The effect of 17β-estradiol on the AMPA receptor was then examined, and we observed potentiation of the AMPA component by 17β-estradiol in 5 of 14 cells (36%) (Fig. 2, A and B). This result is consistent with a previous report (Wong and Moss 1991).

17β-estradiol enhancement of LTP

Consistent with the results of intracellular studies, an increase in synaptic transmission occurred in CA1, after acute perfusion of 100 pM 17β-estradiol onto experimental hippocampal slices (fEPSP mean increase of slope was 113% (experimental) vs. 101% (control); fEPSP mean increase of amplitude was 112% (experimental) vs. 100% (control), F(1,180) = 229, P < 0.0001, and F(1,180) = 353, P < 0.0001, respectively). Field EPSP responses of the two groups were identical during the baseline period before 17β-estradiol perfusion (fEPSP slope and fEPSP amplitude, F(1,58) = 0.000, NS, and F(1,58) = 0.000, NS). In Fig. 3, the two groups of
slices from normal, young adult male rats (17β-estradiol-treated vs. aCSF-control) showed no significant differences in either their measured fEPSP slopes or fEPSP amplitudes over the 10-min baseline period. The increase in synaptic transmission began to develop ~3–4 min after 17β-estradiol perfusion onto the experimental slices and continued throughout the 30-min perfusion period.

When LTP was assessed after high-frequency stimulation, fEPSP slopes and amplitudes were increased significantly for the 17β-estradiol–treated slices compared with control slices. fEPSP mean increase of slope was 192% (experimental) vs. 154% (control); fEPSP mean increase of amplitude was 176% (experimental) vs. 156% (control), $F(1,200) = 305.86$, $P < 0.0001$, and $F(1,200) = 113.58$, $P < 0.0001$, respectively. Thus hippocampal slices treated with 17β-estradiol exhibited a pronounced, persisting, and significant increase in LTP as measured by both population fEPSP slope and fEPSP amplitude recordings.

**DISCUSSION**

These experiments establish several fundamental characteristics of the effects of estrogen on synaptic transmission in the mammalian CNS. First, we demonstrate that estrogen acts rapidly via presumed membrane mechanisms to enhance both NMDA and AMPA receptor/channel processes in response to glutamate released from Schaffer collateral terminals. Wong and Moss (1992) reported that estradiol enhances the amplitude of EPSPs at the Schaffer collateral-CA1 synapse. They observed that the increase in EPSP amplitude remains unchanged in the presence of D-APV but was blocked by CNQX, suggesting the NMDA receptor irrelevant in regard to the effects of estradiol on synaptic transmission. However, even for AMPA receptor-mediated EPSPs, the enhancing effect of estradiol was only seen in 36% of their cells. Furthermore, the NMDA component accounts for only a small fraction of the total EPSP profile under their experimental conditions. Consequently, in such conditions, any effect of estradiol on NMDA receptor/channel function would be minimally expressed and may be undetectable. In fact, in our experiments under conditions in which a lowered extracellular concentration of Mg$^{2+}$ was applied, estradiol led to a higher percentage enhancement of the NMDA component compared with the AMPA component (75 vs. 36%). The experimental conditions used here, i.e., pharmacological isolation of the two types of receptors, also ruled out the possibility of the enhancement of one receptor component as the result of the other. Thus estradiol seems to act on both NMDA and AMPA receptors and produce acute effects in a similar fashion. It seems unlikely that these effects are presynaptic; estrogen can induce an increase in the number of stimulus-evoked action potentials in the Schaffer collaterals or an increase in the amount of glutamate released per Schaffer collateral action potential, but this possibility was not definitely ruled out here or in earlier studies. It is not yet known whether these estrogen effects are due to an action directly on the receptors or indirectly via second messenger processes that in turn influence NMDA and AMPA receptor/channel processes.

Second, our results indicate that estradiol can both increase synaptic transmission in the hippocampus and markedly enhance LTP in CA1 neurons of adult, male rats. The enhancement of LTP after acute 17β-estradiol application (Fig. 3) could be due to an increase in activation of NMDA receptors/
channels or an increase in AMPA receptor function. Both possibilities are consistent with our intracellular data. Whatever the mechanism, the fact is that estrogen enhances LTP in hippocampal CA1. To the extent that LTP is a mechanism involved in processes of coding and storage of information, i.e., in memory formation, estrogen enhances these processes. Indeed, the estrogen enhancement of LTP reported here suggests a possible mechanism whereby estrogen can exert its facilitatory effects on memory processes in humans. Recent clinical evidence indicates that estrogenic steroids can enhance cognitive functions in humans, particularly in postmenopausal women (Henderson et al. 1997; Kawas et al. 1997). Although the estrogen regulation of functions within the limbic system may result mostly from the classical genomic mechanism, an acute, nongenomic effect of estrogen could provide an additional short-term mechanism in modulating synaptic transmission and plasticity. Our studies demonstrate that estradiol enhances synaptic transmission through both NMDA and AMPA receptors/channels and that these enhancements may underlie its facilitatory effect on the magnitude of LTP.

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