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Long-term and Short-term Electrophysiological Effects of Estrogen on the Synaptic Properties of Hippocampal CA1 Neurons

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The ovarian steroids exert both long-term and short-term actions on neurons involving different cellular mechanisms. We have investigated the long-term and short-term effects of estrogen on the electrophysiological properties of CA1 neurons utilizing intracellular recordings in hippocampal slices prepared from ovariectomized female rats. An *in vivo* estrogen-priming paradigm was used to examine long-term genomic actions of estrogen. Subcutaneous estrogen injections 2 d prior to recording had no effect on the intrinsic membrane properties of CA1 neurons, but increased synaptic excitability by prolonging the EPSP and inducing repetitive firing in response to Schaffer collateral stimulation. Short-term effects of estrogen that presumedly involve direct membrane interactions were tested by application of steroids directly to the slice. Superfusion of 17β-estradiol, but not 17α-estradiol, caused a rapid and reversible increase in the amplitude of the Schaffer collateral–activated EPSP. This potentiation of the EPSP by 17β-estradiol still occurred in the presence of the NMDA antagonist 2-amino-5-phosphonovalerate, but was blocked by the non-NMDA antagonist 6-cyano-7-nitroquinoxaline-2,3-dione. Depolarizing responses to iontophoretic pulses of exogenous glutamate were also potentiated by 17β-estradiol, suggesting a postsynaptic site of action. In addition, 17β-estradiol potentiated the responses to α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid, kainate, and quisqualate, but not NMDA, further implicating non-NMDA receptors in the short-term action of estrogen. In contrast, 17β-estradiol had no effect on responses to exogenous GABA or on the Schaffer collateral–induced late IPSP. These findings indicate that synaptic functioning and neuronal excitability in the hippocampus are subject to both long-term and short-term excitatory modulation by estrogen.

Steroid hormones have a variety of morphological, biochemical, and electrophysiological effects on neurons in a number of different regions in mammalian brain (for reviews, see McEwen and Parsons, 1982; Erulkar and Wetzel, 1987; McEwen, 1991). The neural actions of steroids can be categorized generally into two cellular mechanisms. In the classical genomic mechanism, steroids exert long-term effects with time courses lasting hours to days by activating intracellular receptors that regulate transcription and protein synthesis. In the more novel nongenomic mechanism, steroids have very rapid, short-term effects that are most likely due to direct interactions with neural membranes and in some cases have been shown to involve specific membrane receptors (Majewska et al., 1986; Orchinik et al., 1991). Many of the long-term genomic and short-term membrane actions of steroids influence synaptic transmission and functioning. In particular, the ovarian steroids, estrogens and progestins, have been found to regulate the excitatory and inhibitory neurotransmitter systems in the hippocampus.

The classical intracellular estrogen receptor has been identified in the hippocampus by several different methods (Pfaff and Keiner, 1973; Loy et al., 1988; Pelletier et al., 1988; Maggi et al., 1989). Estrogen has a number of long-term genomic effects, which influence synaptic transmission at the Schaffer collateral–CA1 synapse, with time courses on the order of days. Two days following subcutaneous estrogen injections, the binding of specific receptor subtypes of glutamate and GABA, the major excitatory and inhibitory neurotransmitters in the hippocampus, is increased in the CA1 region (Schumacher et al., 1989; Weiland, 1990). Furthermore, the density of CA1 dendritic spines, which represent the primary site of innervation for glutaminergic synapses, increases with estrogen priming and fluctuates across the estrous cycle of the female rat (Gould et al., 1990; Woolley et al., 1990). Estrogen priming also lowers seizure threshold in the hippocampus (Terasawa and Timiras, 1968) and increases expression of c-*fos* in the CA1 region (Jennes, 1990), further indicating that estrogen has long-term activational effects on hippocampal physiology.

The female sex steroids also have rapid nongenomic effects on CA1 neurons relevant to synaptic transmission, with time courses on the order of seconds to minutes. The progesterone metabolite 3α-hydroxy-5α-dihydroprogesterone (3α-OH-DHP) binds specifically to the $GABA_A$ receptor to potentiate the GABA-activated chloride current in CA1 neurons (Majewska et al., 1986). While this progesterone metabolite–GABA interaction is presently one of the best-studied examples of a membrane effect of steroids, evidence also exists that estrogens may have a similar action on glutamate receptors (Smith et al., 1987, 1988). In the hippocampal slice preparation, bath application of 17β-estradiol increases the extracellular CA1 field potential in response to stimulation of the Schaffer collaterals, which make glutaminergic synapses onto CA1 neurons (Teyler et al., 1980). Using intracellular recording techniques, we have previously reported that 17β-estradiol triggers a rapid depolarization in a small percentage of CA1 neurons (Wong and Moss, 1991). In

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Table 1. Membrane properties of CA1 neurons from ovariectomized nonprimed and estrogen-primed rats

Treatment (cells)	Resting potential (mV)	Input resistance (MΩ)	Time constant (msec)	Distance to threshold (mV)	Action potential		After hyperpolarization		Repetitive firing (spikes)
					Amplitude (mV)	Half-duration (msec)	Amplitude (mV)	Half-duration (msec)	
OVX (n = 76)	60.5 ± 0.5	51.4 ± 2.1	10.5 ± 0.2	10.4 ± 0.3	63.5 ± 0.7	1.2 ± 0.1	5.1 ± 0.2	481.8 ± 38.8	5.6 ± 0.4
OVX + EB (n = 74)	60.6 ± 0.4	48.6 ± 1.9	10.4 ± 0.3	10.0 ± 0.3	65.1 ± 0.7	1.2 ± 0.1	4.9 ± 0.2	498.5 ± 45.5	5.0 ± 0.3

Values are means ± SEM. Action potential amplitude was measured from threshold to peak. Afterhyperpolarization values were obtained following 0.5 nA, 50 msec depolarizing current pulses. The number of spikes with repetitive firing was measured during 0.5 nA, 500 msec depolarizing pulses. OVX, neurons from ovariectomized non-primed rats; OVX + EB, neurons from ovariectomized estrogen-primed rats.

the present study, we provide evidence that *in vitro* application of estrogen also induces a more prevalent short-term facilitation of glutaminergic transmission at the Schaffer collateral–CA1 synapse. In addition, *in vivo* estrogen priming is also shown to have long-term excitatory effects on synaptic function in the hippocampus. These findings indicate that estrogen can utilize different cellular mechanisms within a single group of neurons to regulate neuronal and synaptic excitability.

Materials and Methods

Seventy-one adult female Sprague–Dawley rats (200–300 gm) were used in these experiments. All animals were ovariectomized under ether anesthesia at least 2 weeks prior to use. Some of the animals were primed with subcutaneous injections of 10 μg of estrogen benzoate in 0.05 ml of corn oil on both the third and second day prior to an experiment (n = 33), while the remaining rats received vehicle injections (n = 38). On the day of an experiment, hippocampal slices (400–450 μm thick) were prepared using a Lancer vibratome and placed in a holding chamber containing an oxygenated (95% O_2, 5% CO_2) artificial cerebrospinal fluid (ACSF). Individual slices were transferred to a Haas interface recording chamber, where they were continuously superfused with the oxygenated ACSF at 33°C and humidified with O_2/CO_2 gas passed over the surface of the slice. The composition of the ACSF was (in mM) NaCl (126), KCl (5), $CaCl_2$ (2.5), $MgCl_2$ (1.3), $Na_2H_2PO_4$ (1.25), $NaHCO_3$ (26), and glucose (10).

Conventional intracellular recordings were made from CA1 neurons in the stratum pyramidale using borosilicate glass electrodes (70–130 MΩ) filled with 4 M potassium acetate. Voltage signals from the neuron were passed through a Medical Systems intracellular bridge amplifier and displayed and stored on a Nicolet 3100 digital oscilloscope, Gould TA4000 chart recorder, and AT&T 6300 computer with a custom software program. Depolarizing and hyperpolarizing current pulses were injected into the cell to test a number of passive and active membrane properties of the neuron. Only cells with resting membrane potentials of at least −55 mV, input resistances of at least 20 MΩ, and overshooting action potentials were included in this study. In some experiments, a bipolar metal stimulating electrode was placed in the stratum radiatum to activate Schaffer collateral synaptic inputs with 0.1 msec current pulses (10–500 μA) at 0.1 Hz. In other experiments, a multibarreled glass electrode filled with agonists of glutamate and GABA was placed in the stratum radiatum just adjacent to the recording electrode. The following drugs were made up in distilled water for iontophoretic application: glutamate (0.1 M, pH 8.5; Sigma), GABA (0.1 M, pH 6.5; Sigma), α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA; 0.01 M, pH 8.5; Sigma), kainate (0.01 M, pH 8.5; Sigma), quisqualate (0.01 M, pH 8.5; Sigma), *N*-methyl-D-aspartate (NMDA; 0.01 M, pH 8.5; Tocris Neuramin). One barrel containing ACSF was used for current balancing, and retaining currents of 3–5 nA of the appropriate polarity were used on the drug barrels. Drugs could also be applied to the bath by switching a stopcock in the superfusion system. The following drugs were made up in the ACSF for bath application: 17β-estradiol (10^{-10}–10^{-8} M; Sigma), 17α-estradiol (10^{-10}–10^{-8} M; Sigma), 3α-OH-DHP (1 μM; Sigma), kynurenic acid (1 mM; Sigma), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10 μM; Tocris Neuramin), 2-amino-5-phosphonovalerate (AP5; 50 μM; Tocris Neuramin). The estrogens were initially dissolved in a small amount of ethanol and diluted by ACSF to a final ethanol concentration of 0.001% at a steroid concentration of 10^{-8} M.

The following procedure was used to assess the short-term effects of estrogen on synaptic responses to Schaffer collateral stimulation and responses to iontophoretically applied glutamate agonists. In control conditions, brief synaptic or iontophoretic stimulation, applied repetitively at low frequency (0.1 Hz), was set at an intensity level to elicit very consistent EPSPs that were approximately half the distance to threshold, and thus never elicited action potentials. Keeping the stimulation parameters constant throughout the remainder of testing, estrogens were bath applied to determine the effect on these subthreshold responses. In all cases where estrogen appeared to be effective, the subthreshold responses were potentiated to a suprathreshold level.

Results

Long-term effects of estrogen priming

A number of previous studies have described in detail the intrinsic and synaptic electrophysiological characteristics of CA1 neurons (Kandel and Spencer, 1961; Kandel et al., 1961; Spencer and Kandel, 1961a,b; Schwartzkroin, 1975, 1977), but without reference to the sex or estrogen status of the animals. In the present study, comparisons were made between CA1 neurons from ovariectomized nonprimed and estrogen-primed female rats to determine the long-term genomic effects of estrogen priming on a number of membrane and synaptic properties. Table 1 lists the average values for the passive and active membrane properties of 150 CA1 neurons that satisfied the criteria for inclusion in this study. There were no significant differences between CA1 neurons from nonprimed and estrogen-primed rats in any of the intrinsic membrane properties measured, including resting membrane potential, input resistance, distance to threshold, repetitive firing, and afterhyperpolarization.

The synaptic response of CA1 neurons to Schaffer collateral stimulation has previously been characterized as a brief glutamate-mediated EPSP, which typically triggers only a single action potential upon reaching threshold, followed by a longer-lasting GABA-mediated IPSP. Figure 1 shows examples of synaptic responses of CA1 neurons from nonprimed and estrogen-primed rats to Schaffer collateral stimulation of increasing intensity. In all 60 neurons tested from nonprimed animals, Schaffer collateral stimulation could trigger an EPSP with only a single action potential, regardless of how high the stimulation intensity was increased (Fig. 1*A*). In contrast, 15 of 62 neurons from estrogen-primed animals responded to increasing Schaffer collateral stimulation with repetitive action potentials (Fig. 1*B*), representing a significant difference from the nonprimed group (χ^2 = 16.6, 1 df, $p < 0.001$). Table 2 summarizes the data for a number of parameters of the evoked EPSP and IPSP. For the

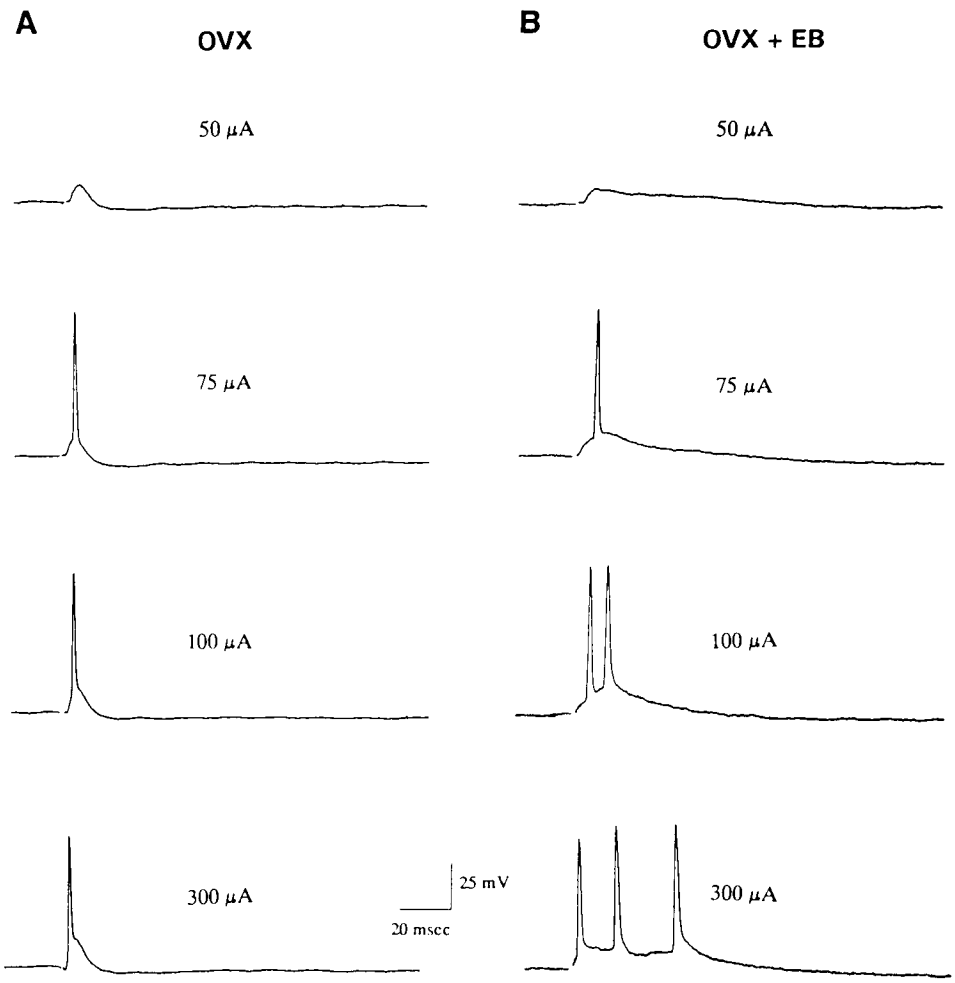

Figure 1. Synaptic responses of CA1 neurons from ovariectomized nonprimed and estrogen-primed rats to Schaffer collateral stimulation. *A*, In all CA1 neurons from ovariectomized nonprimed rats (*OVX*), increasing the intensity of Schaffer collateral stimulation increased the duration and initial slope of the EPSP, but could only trigger a single action potential. *B*, In some CA1 neurons from estrogen-primed rats (*OVX + EB*), a long-lasting EPSP and multiple action potentials could be elicited with increasing Schaffer collateral stimulation.

purpose of comparing between the two animal groups, measurements were made at a stimulation level that induced a just-subthreshold EPSP. The average duration of EPSPs of neurons from estrogen-primed rats was significantly greater than neurons from nonprimed animals. There was no significant difference in the duration or amplitude of the IPSP.

Short-term effects of estrogen

The short-term direct membrane effects of estrogen on synaptic responses of CA1 neurons to Schaffer collateral stimulation were tested by bath applying the steroid directly to hippocampal slices from both nonprimed and estrogen-primed rats. In the few cells that were depolarized by direct estrogen application (see below), the membrane potential was returned to control levels by injecting constant hyperpolarizing current to test the synaptic response. In 35 out of 64 neurons tested (25 of 35 from estrogen-primed, 10 of 29 from nonprimed animals), 17β-estradiol at a concentration of 10^{-8} M induced an increase in the size of the EPSP, causing a previously subthreshold EPSP to reach threshold (Fig. 2*A,C,D*). In contrast, the inactive control analog, 17α-estradiol, tested on the same neurons, potentiated the EPSP in none of the 64 cells (Fig. 2*B*; $\chi^2 = 48.2$, 1 df, $p < 0.001$ in comparing treatments with 17β- and 17α-estradiol). While 17β-estradiol increased the EPSP duration only by an average of 22 ± 2%, the EPSP amplitude was potentiated by 90 ± 3% to reach threshold. This potentiation of the EPSP by 17β-estradiol occurred consistently with a latency of less than 2 min and was reversible shortly after washout of the drug (Fig. 2*E*). A signif-

Table 2. Synaptic properties of CA1 neurons from ovariectomized nonprimed and estrogen-primed rats

	EPSP				IPSP		
Treatment (cells)	Peak amplitude (mV)	Base duration (msec)	Half-duration (msec)	10–90% rise time (msec)	Peak amplitude (mV)	Half-duration (msec)	10–90% rise time (msec)
OVX ($n = 60$)	8.2 ± 0.4	12.0 ± 0.6	6.5 ± 0.3	3.3 ± 0.1	5.2 ± 0.3	61.4 ± 4.9	10.8 ± 0.5
OVX + EB ($n = 62$)	9.0 ± 0.3	21.2 ± 2.2*	10.4 ± 1.0*	3.7 ± 0.2	4.6 ± 0.4	65.8 ± 6.6	11.8 ± 0.6

Values are means ± SEM. The Schaffer collaterals were stimulated at an intensity to elicit just-subthreshold EPSPs. OVX, neurons from ovariectomized nonprimed rats; OVX + EB, neurons from ovariectomized estrogen-primed rats.

* $p < 0.001$ for comparisons between treatment groups with Student's t test.

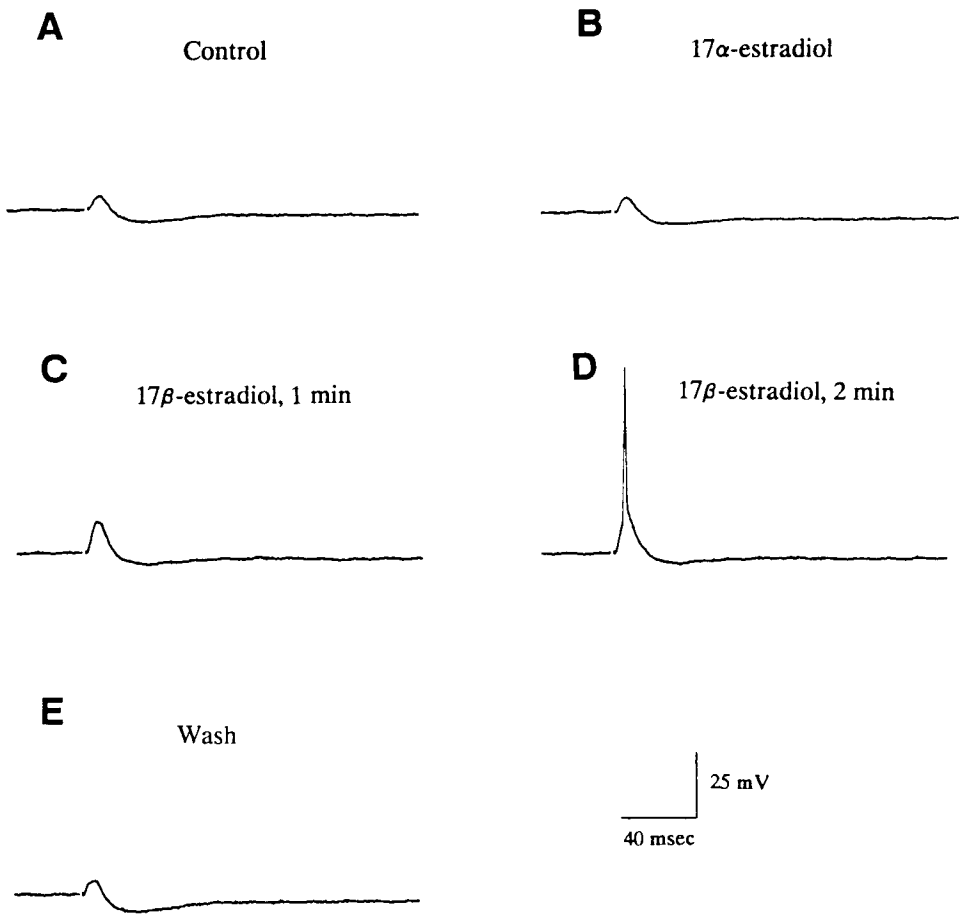

Figure 2. 17β-Estradiol, but not 17α-estradiol, potentiates the EPSP of CA1 neurons. *A*, In control conditions, the Schaffer collaterals were stimulated with 0.1 msec, 50 μA pulses at a frequency of 0.1 Hz to elicit consistent subthreshold EPSPs. The stimulation parameters were kept constant throughout the remainder of testing. *B*, Bath application of 10^{-8} M 17α-estradiol for 2 min had no effect on the EPSP. The trace shown was sampled at 2 min of 17α-estradiol application. *C* and *D*, Bath application of 10^{-8} M 17β-estradiol caused the EPSP to increase in amplitude and reach threshold with a latency of less than 2 min. *E*, Washout of the 17β-estradiol returned the EPSP to control size. All records were taken from the same CA1 neuron from an estrogen-primed animal.

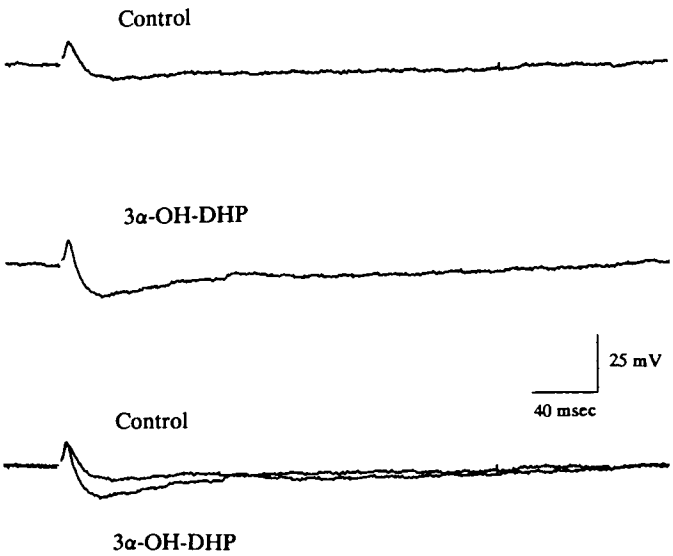

Figure 3. 3α-OH-DHP potentiates the IPSP of CA1 neurons. Records show examples of synaptic responses to Schaffer collateral stimulation (0.1 msec, 30 μA, 0.1 Hz) before (control) and during a 2 min bath application of 1 μM 3α-OH-DHP. 3α-OH-DHP increases the amplitude and duration of the IPSP following the EPSP. Both records were taken from the same neuron from a nonprimed animal.

icantly larger proportion of neurons from estrogen-primed animals (25 of 35) exhibited the short-term synaptic facilitation by 17β-estradiol compared to neurons from nonprimed animals (10 of 29), indicating that the long-term estrogen status of the animal can influence the rapid action of estrogen ($\chi^2 = 8.9$, 1 df, $p < 0.005$). In contrast to the EPSP, 17β-estradiol had no significant effect on the late IPSP. In comparison, 3α-OH-DHP, an ovarian steroid that binds to the $GABA_A$ receptor to facilitate GABA-activated chloride currents, could potentiate the IPSP in 17 of 19 cells (Fig. 3), serving as a positive control for the 17β-estradiol. Estrogen priming *in vivo* did not influence the rapid action of 3α-OH-DHP, as the progesterone metabolite potentiated the IPSP in 10 of 11 cells from nonprimed animals and 7 of 8 cells from estrogen-primed animals.

The glutamate antagonists CNQX and AP5 were bath applied separately to neurons that displayed the synaptic potentiation by 17β-estradiol to determine whether specific receptor subtypes for glutamate were involved in the short-term synaptic effects of estrogen. Application of the NMDA antagonist AP5 at a concentration that has previously been shown to block NMDA-mediated currents (50 μM; Hestrin et al., 1990) had little or no effect on the EPSP evoked by low-frequency (0.1 Hz) stimulation of the Schaffer collaterals (Fig. 4*A*), indicating that non-NMDA currents were mediating most of the EPSP under these conditions. In the presence of AP5, 17β-estradiol could still potentiate the EPSP in five of five cells tested, suggesting that non-NMDA glutamate receptors are sensitive to the facilitating effect of es-

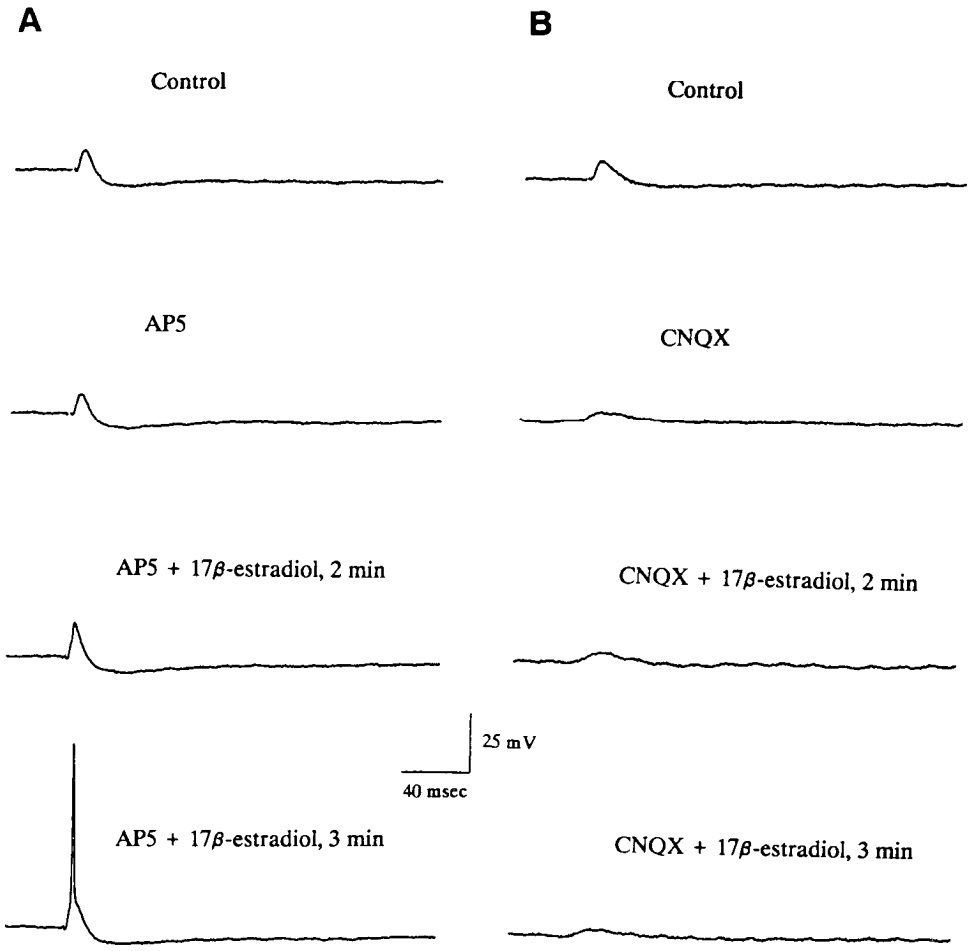

Figure 4. Synaptic potentiation by 17β-estradiol involves a non-NMDA component of the EPSP. The effect of AP5 and CNQX on synaptic responses of two different neurons that had exhibited synaptic potentiation by 17β-estradiol is shown. In control conditions, the Schaffer collaterals were stimulated with 0.1 msec, 50 μA pulses at a frequency of 0.1 Hz to elicit consistent subthreshold EPSPs. *A*, In this neuron from an estrogen-primed animal, bath application of 50 μM AP5 had no significant effect on the Schaffer collateral–stimulated EPSP. In the presence of AP5, application of 17β-estradiol could still potentiate the EPSP. *B*, In another neuron from an estrogen-primed animal, application of 10 μM CNQX significantly reduced the EPSP, leaving a small, presumedly NMDA component. This residual component could not be potentiated by subsequent application of 17β-estradiol.

trogen. In contrast, application of the non-NMDA antagonist CNQX (10 μM) blocked most or all of the EPSP (Fig. 4*B*). In the presence of CNQX, estrogen could not potentiate any residual EPSP in four of four trials, indicating that NMDA receptors are not involved in estrogen-induced facilitation at least under conditions of low-frequency synaptic transmission. Furthermore, since the estrogen-induced facilitation appeared to be selective for the non-NMDA component of glutaminergic trans-

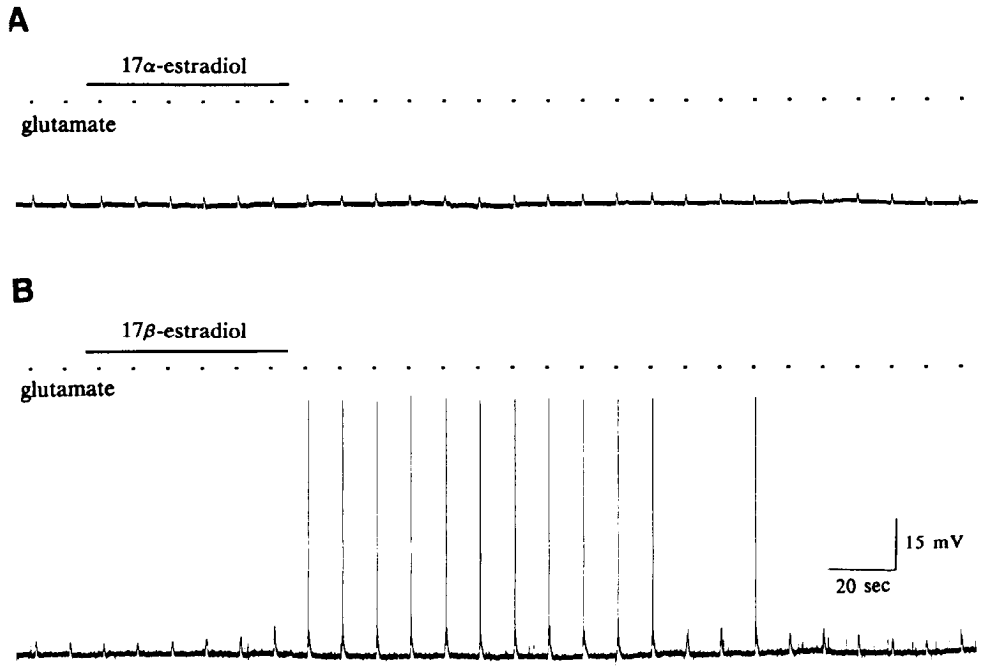

Figure 5. 17β-Estradiol, but not 17α-estradiol, potentiates responses to exogenous glutamate. Glutamate was applied to the cell from an iontophoretic electrode with 1 sec pulses at 0.1 Hz (*dots*) to elicit consistent subthreshold depolarizations. 17β-Estradiol (*A*) or 17β-estradiol (*B*) at 10^{-8} M was applied through the superfusion system for 1 min (*bars*). There is approximately a 1 min lag time in arrival of the steroid to the tissue. 17α-Estradiol had no effect on depolarizing responses to glutamate, but 17β-estradiol potentiated subthreshold depolarizations to a suprathreshold level. Both records are from the same neuron from an estrogen-primed animal.

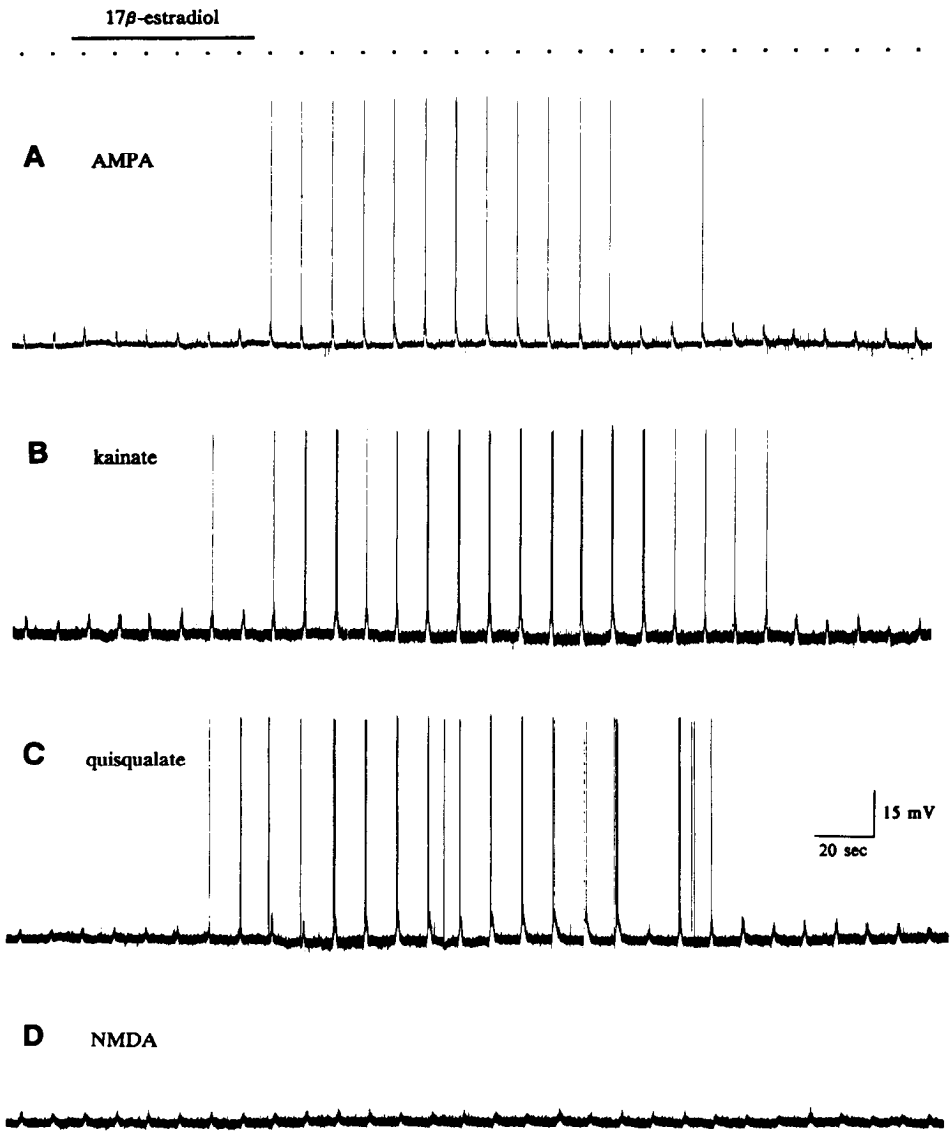

Figure 6. 17β-Estradiol potentiates responses to non-NMDA agonists, but not to NMDA. The specific glutamate agonists listed were applied to the cell from an iontophoretic electrode with 1 sec pulses at 0.1 Hz (*dots*) to elicit consistent subthreshold depolarizations. 17β-Estradiol at 10^{-8} M was applied through the superfusion system for 1 min (*bar*). There is approximately a 1 min lag time in arrival of the steroid to the tissue. 17β-Estradiol potentiated subthreshold depolarizing responses to AMPA, kainate, and quisqualate, but not NMDA. Records *B–D* are from the same neuron from a nonprimed animal, and *A* is from a different neuron from an estrogen-primed animal.

mission, this suggests that estrogen is acting postsynaptically on specific glutamate receptor subtypes, not presynaptically on glutamate release.

To address further the issue of whether the action of estrogen on synaptic transmission involved a presynaptic or postsynaptic mechanism, the effect of estrogen was tested on responses to exogenously applied glutamate agonists. Subthreshold depolarizations induced by iontophoretic pulses of glutamate were potentiated to suprathreshold levels by bath application of 17β-estradiol, but not 17α-estradiol, in five of six cells tested (98 ± 10% increase in amplitude), again suggesting that estrogen is acting postsynaptically (Fig. 5). To test for the involvement of specific receptor subtypes for glutamate, the glutamate agonists AMPA, kainate, quisqualate, and NMDA were used. 17β-Estradiol potentiated the responses to the non-NMDA agonists quisqualate in three of four cells, AMPA in three of three cells, and kainate in two of three cells (Fig. 6), but had no effect on responses to NMDA (none of four cells). These results again implicate non-NMDA receptors in the rapid action of estrogen. The potentiation of subthreshold responses to glutamate agonists by 17β-estradiol occurred consistently with a latency of less than a minute.

As the late portion of the EPSP is normally opposed by the subsequent IPSP, the possibility that estrogen might be enhancing the EPSP secondarily by suppressing the IPSP was considered by testing responses to exogenous GABA. 17β-Estradiol had no effect (n = 5) on the hyperpolarizing responses to iontophoretic GABA (Fig. 7*A*), suggesting that estrogen is acting solely on the glutaminergic EPSP and not the GABAergic IPSP. In contrast, 3α-OH-DHP could enhance GABA-induced hyperpolarizations (four of four cells; Fig. 7*B*).

Consistent with a previous study (Wong and Moss, 1991), bath application of 17β-estradiol induced a rapid depolarization in a small percentage (12 of 75) of CA1 neurons in this study. A possible explanation for this occasional depolarization is that estrogen was again affecting glutaminergic mechanisms, either to potentiate the action of endogenous glutamate in the tissue or to activate the glutamate receptor channel directly. In four of four trials, however, the glutamate antagonist kynurenic acid was not capable of blocking estrogen-induced depolarizations

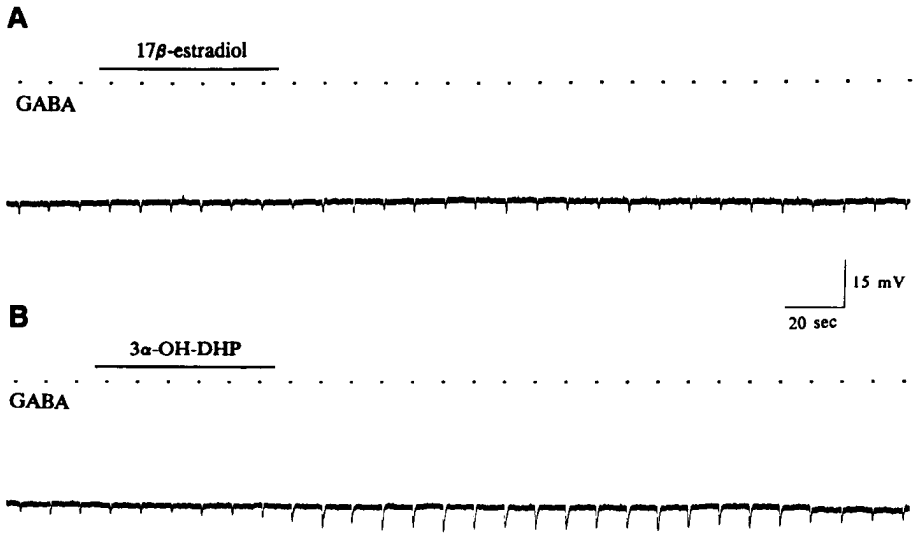

Figure 7. 3α-OH-DHP, but not 17β-estradiol, potentiates responses to exogenous GABA. GABA was applied to the cell from an iontophoretic electrode with 1 sec pulses at 0.1 Hz (*dots*) to elicit consistent hyperpolarizations. 17β-Estradiol at 10^{-8} M (*A*) or 3α-OH-DHP at 10^{-6} M (*B*) was applied through the superfusion system for 1 min (*bars*). There is approximately a 1 min lag time in arrival of the steroid to the tissue. 17β-Estradiol had no effect on hyperpolarizing responses to GABA, but 3α-OH-DHP potentiated GABA-induced hyperpolarizations. Both records are from the same neuron from an estrogen-primed animal.

(data not shown), suggesting that estrogen may directly activate a glutamate receptor at a site insensitive to glutamate antagonists or that a completely separate mechanism may be operating.

Discussion

Long-term effects of estrogen

Estrogen has previously been shown to have a number of genomic effects on the morphological and biochemical properties of hippocampal neurons that would predict an excitatory action of this steroid on synaptic physiology in the hippocampus. In particular, estrogen priming increases NMDA binding sites in the CA1 region (Weiland, 1990) and the density of CA1 dendritic spines, which receive excitatory synapses (Gould et al., 1990; Woolley et al., 1990). In the present study, we examined the effects of estrogen priming on the intracellular electrophysiological properties of CA1 neurons. While the intrinsic membrane properties of these cells were not altered, in some CA1 neurons estrogen priming appeared to increase the duration of the EPSP and induce repetitive firing in response to Schaffer collateral stimulation. The finding that only a small proportion of neurons from estrogen-primed animals displayed this characteristic may be consistent with the relatively low amounts of intracellular estrogen receptors in the hippocampus (Pfaff and Keiner, 1973; Loy et al., 1988). Prolongation of the EPSP and induction of repetitive firing are suggestive of either the potentiation of a slow NMDA component of the EPSP or a reduction in the disynaptic IPSP, which opposes the late part of the EPSP. The former possibility seems more likely, as there was no significant difference in the duration or amplitude of the IPSP between the two groups. Furthermore, previous studies have shown that estrogen priming appears to increase, not decrease, GABA binding (Schumacher et al., 1989), as well as NMDA binding (Weiland, 1990), in the CA1 region.

The potentiated EPSP with multiple action potentials in some neurons from estrogen-primed animals resembles bursting responses observed in models of epileptiform activity in the hippocampus. The epileptogenic potential of estrogen is supported by an early study showing that estrogen priming decreases seizure threshold in rat hippocampus (Terasawa and Timiras, 1968). Furthermore, estrogen priming stimulates the expression of c-*fos* in the CA1 (Jennes, 1990), a marker that has been correlated with seizure-like activity, and increases the membrane and synaptic excitability of neurons of the amygdala, another region with a low seizure threshold (Schiess et al., 1988). Finally, the steroidal modulation of synaptic excitability may represent a general mechanism with direct clinical relevance, as the incidence of seizures in women with certain types of epilepsy has been correlated with the fluctuating estrogen levels across the menstrual cycle (Backstrom, 1976).

Short-term effects of estrogen

In addition to the long-term genomic actions, estrogen, as well as a variety of other steroids, has been shown to exert very fast electrophysiological effects that presumedly involve direct membrane mechanisms. *In vivo* experiments involving local application of 17β-estradiol to extracellularly recorded preoptic/septal neurons gave the first direct evidence that estrogen could rapidly modulate the electrical activity of neurons (Kelly et al., 1976, 1977). Since then, a number of studies utilizing various techniques and preparations from many regions of the brain have revealed additional examples of immediate electrophysiological actions of estrogen and have identified possible membrane mechanisms used by the steroid. In the amygdala and ventromedial hypothalamus, rapid changes in membrane potential by 17β-estradiol have been attributed to the modulation of potassium channels (Nabekura et al., 1986; Minami et al., 1990), and some of these changes may involve the second messenger cAMP (Minami et al., 1990). In contrast, work in the cerebellum indicates that estrogen may directly enhance the function of receptors for excitatory amino acids (Smith et al., 1987, 1988).

Extracellular field potential recordings from the hippocampal slice preparation also suggest that estrogen can rapidly facilitate glutaminergic neurotransmission (Teyler et al., 1980). While the initial studies reported that the potentiation of the CA1 population spike by 17β-estradiol was selective for male rats, others have found a similar effect in females (Landgren, 1992). The present study investigated the possibility of an estrogen–glutamate interaction in more detail at the single neuron level and indeed observed a fast potentiation of the EPSP in individual CA1 neurons by 17β-estradiol in response to Schaffer collateral stimulation. Since estrogen could also potentiate both the EPSP in the presence of AP5 and the responses to exogenously applied non-NMDA agonists, this study provides evidence that estrogen

is acting postsynaptically on non-NMDA receptors. Potentiation of a slow NMDA component or suppression of the GABAergic IPSP are also possible explanations for the observed short-term synaptic facilitation, but these possibilities seem unlikely as there was no dramatic increase in the duration of the EPSP and estrogen had no effect on synaptic responses in the presence of CNQX or on iontophoretic responses to NMDA or GABA. Furthermore, the NMDA component appears to contribute little or no part of the normal EPSP during low-frequency synaptic stimulation (Fig. 3; Collingridge et al., 1983, 1988). However, the possibility that estrogen exerts a rapid effect on NMDA receptors during high-frequency stimulation cannot be ruled out.

While the effects of estrogen priming have slow time courses spanning several days and presumedly involve genomic mechanisms that secondarily alter synaptic functioning, the rapid and reversible nature of the effect of bath application of 17β-estradiol strongly indicates a direct membrane action. In facilitating responses to glutamate, estrogen might bind directly to a membrane protein associated with or part of a glutamate receptor or perturb the lipid bilayer to influence the glutamate receptor indirectly. Consistent with reports of other rapid actions of estrogen (Kelly et al., 1977; Foy and Teyler, 1983; Smith et al., 1988), the lack of effect of 17α-estradiol compared to 17β-estradiol in this study demonstrates the stereospecificity of the response, providing strong, but not conclusive evidence for a direct protein interaction. While specific binding for estrogen on glutamate receptors has not yet been demonstrated, the discovery of a high-affinity steroid binding site on the $GABA_A$ receptor (Majewska et al., 1986) makes such a possibility seem plausible.

Interactions between long-term and short-term effects of estrogen

Overall, estrogen appears to utilize two distinct cellular mechanisms with vastly different time courses to facilitate excitatory synaptic transmission in the hippocampus in a similar, but not identical, fashion. A 2 d estrogen priming paradigm that activates genomic mechanisms prolonged the EPSP and induced repetitive firing in some CA1 neurons. Bath application of 17β-estradiol increased a non-NMDA component of the EPSP within 2 min, indicating a direct membrane action. It has previously been hypothesized that genomic and nongenomic mechanisms of action of steroids might operate within the same neuron and might even interact through the same molecular target (Schumacher, 1990; McEwen, 1991). This study presents evidence that both a genomic and a direct membrane mechanism can indeed be activated in CA1 neurons by estrogen. Furthermore, the two mechanisms may converge at the level of the glutamate receptor, although perhaps acting on different receptor subtypes. The finding that the short-term synaptic effects of estrogen were influenced by estrogen priming further supports the interaction of genomic and nongenomic mechanisms. Both actions of estrogen reported in this study have important implications for the role of ovarian steroids in the hormonal regulation of neuronal excitability and epileptiform activity.

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