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Short Communications

Electrophysiological evidence for a rapid membrane action of the gonadal steroid, 17β-estradiol, on CA1 pyramidal neurons of the rat hippocampus

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The rapid electrophysiological effects of 17β-estradiol on CA1 pyramidal neurons (n = 86) were investigated utilizing intracellular recording from the rat hippocampal slice preparation. Bath application of 17β-estradiol, but not 17α-estradiol, caused a reversible depolarization and increased input resistance with a latency of less than 1 min in 19.8% of CA1 neurons tested. There was no significant difference in the percentage of estradiol-responsive cells between male and female rats. Estradiol-responsive cells were identified from prepubertal female rats, as well as females in all stages of the estrous cycle. 17β-estradiol had no effect on the slow afterhyperpolarization or accommodative properties of CA1 neurons. In 2 out of 4 cells tested, the specific antiestrogen, tamoxifen, blocked the excitatory response to 17β-estradiol.

The classical cellular mechanism of steroid action involves diffusion of the steroid through the plasma membrane, binding to cytoplasmic or nuclear receptors, and subsequent regulation of transcription and protein synthesis. In addition to this genomic mechanism, steroids have also been reported to exert rapid, direct actions on neural membranes, which are independent of effects on nuclear transcription (for reviews, see refs. 2, 3, 14, 18). Specific binding sites for corticosterone, estrogen, testosterone, and progesterone have been found associated with synaptic plasma membrane[21]. Furthermore, progesterone metabolites interact specifically with membrane $GABA_A$ receptors[11]. Electrophysiologically, a number of different steroids induce short-term changes in membrane excitability in many regions of the brain[6,13,15,16,20,23].

Estrogen-binding sites have been localized by autoradiography in many parts of the rat brain including preoptic-hypothalamic area, septum, amygdala, and hippocampus[8,12,17]. A number of electrophysiological studies have provided evidence for a direct membrane action of estrogens on nervous tissue. 17β-estradiol induces rapid changes in the electrical excitability and membrane properties of neurons in the hypothalamus[6,13], amygdala[16], and hippocampus[4,20]. Furthermore, a recent report indicates that the depolarizing action of 17β-estradiol on hypothalamic neurons is mediated by cyclic AMP[13]. In the hippocampus, estradiol causes short-term changes in the amplitude of the extracellular CA1 field potential[4,20]. In the present study, we attempt to characterize the rapid effects of estradiol on the electrical properties of individual CA1 neurons, utilizing intracellular recordings from the hippocampal slice preparation. A preliminary report of this work has been presented[25].

Thirty-five Sprague–Dawley rats (9 male, 120–200 g; 26 female, 60–200 g) were used in this study. Daily vaginal smears were taken from the female rats for at least 4 days prior to an experiment to monitor their cycling patterns and to determine the stage of the estrous cycle by microscopic analysis. On the day of an experiment, the rat was classified as prepubertal, diestrus, proestrus, or estrus. Tissue preparation and electrophysiological recording procedures have been described previously[24]. Briefly, hippocampal slices were cut in a transverse plane using a Lancer vibratome and superfused with an oxygenated (95% O_2, 5% CO_2) artificial cerebrospinal fluid (ACSF) composed of (in mM:) NaCl 126, KCl 5, $CaCl_2$ 2.5, $MgSO_4$ 1.3, NaH_2PO_4 1.24, $NaHCO_3$ 26 and glucose 10 at 32 °C.

Intracellular recordings were obtained from CA1 neurons using borosilicate glass microelectrodes (70–120 MΩ) filled with 4 M potassium acetate. Signals were fed through a Medical Systems NL 102 probe and DC amplifier and displayed on a Tektronix 564 oscilloscope, a Nicolet NIC 310 digital oscilloscope, and a Gould 220 chart recorder. Stable intracellular recordings were made

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from 86 CA1 neurons with resting membrane potentials of at least −55 mV and action potential amplitudes of at least 60 mV. Neurons could be depolarized or hyperpolarized by currents injected through the recording electrode. Apparent input resistance was estimated by injecting hyperpolarizing constant current pulses and monitoring the resulting voltage deflection.

Drug solutions of 17α-estradiol and 17β-estradiol (10^{-8} to 10^{-10} M) were prepared in the ACSF with 0.0001% ethanol. Drugs were applied to the tissue for a duration of 2 min by switching a valve in the superfusion system. The lag time between switching of the valve and initial arrival of the drug to the tissue was approximately 1 min. The effect of the biologically active 17β-estradiol on resting membrane potential, apparent input resistance, afterhyperpolarization, and accommodation was tested and compared to that of the weak or inactive analogue, 17α-estradiol, at the same concentration. 17α-estradiol did not affect these properties in any cell tested. In most cells, both estrogen analogues were tested at least twice in random order to test for reproducibility of responses. For some experiments, the estradiol was made up in a calcium-free solution with 10 mM Mg^{2+} and 0.2 mM Cd^{2+} to eliminate synaptic transmission. In other experiments, tamoxifen, an estrogen antagonist, was applied prior to and with 17β-estradiol, in an attempt to block the effect of the estradiol.

Overall, application of 17β-estradiol (Fig. 1B), but not 17α-estradiol (Fig. 1A), induced a reversible depolarization (mean amplitude = 6.8 ± 2.6 mV for 10^{-10} M) in 17 out of 86 (19.8%) CA1 neurons tested. After compensating for the lag time of the arrival of the drug to the tissue, the latency to the onset of this excitatory response was consistently less than a minute. The depolarization was associated with an increase in apparent input resistance (mean % change = 19.2 ± 6.1%) and often with a triggering of action potentials. The increase in input resistance was maintained after returning the membrane potential to control levels by injecting negative background current (–d.c., Fig. 1B). In two neurons

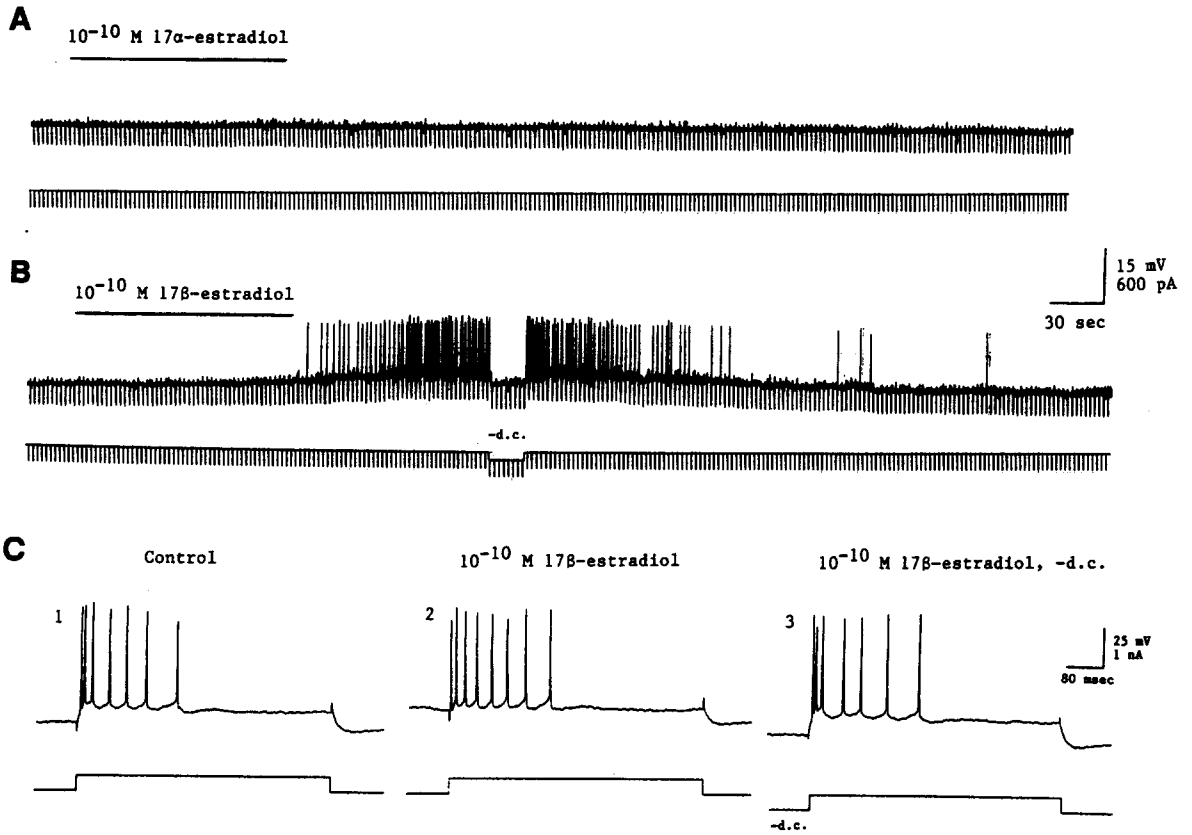

Fig. 1. 17β-estradiol, but not 17α-estradiol, has an excitatory effect on CA1 pyramidal neurons, but does not affect accommodation. All records are from the same cell. The current traces are shown below the voltage traces. The upper calibration scale applies to both A and B, while the lower scale applies to C. In A and B, the horizontal bar denotes a 2-min application of 17α- and 17β-estradiol, respectively, and the downward deflections represent injection of hyperpolarizing current pulses for estimation of input resistance. A: 10^{-10} M 17α-estradiol has no effect on membrane potential or apparent input resistance. B: 10^{-10} M 17β-estradiol induces a reversible depolarization and an increase in apparent input resistance. The increased input resistance is maintained after returning the membrane potential to control levels by injecting negative background current (–d.c.). C: accommodation of repetitive discharge was tested before and immediately after a second applicaton of 17β-estradiol. In control conditions (1), the cell exhibits accommodation. After application of 10^{-10} M 17β-estradiol, the cell is depolarized but still displays accommodation at the depolarized level (2) and after returning the membrane potential to control levels (3, –d.c.).

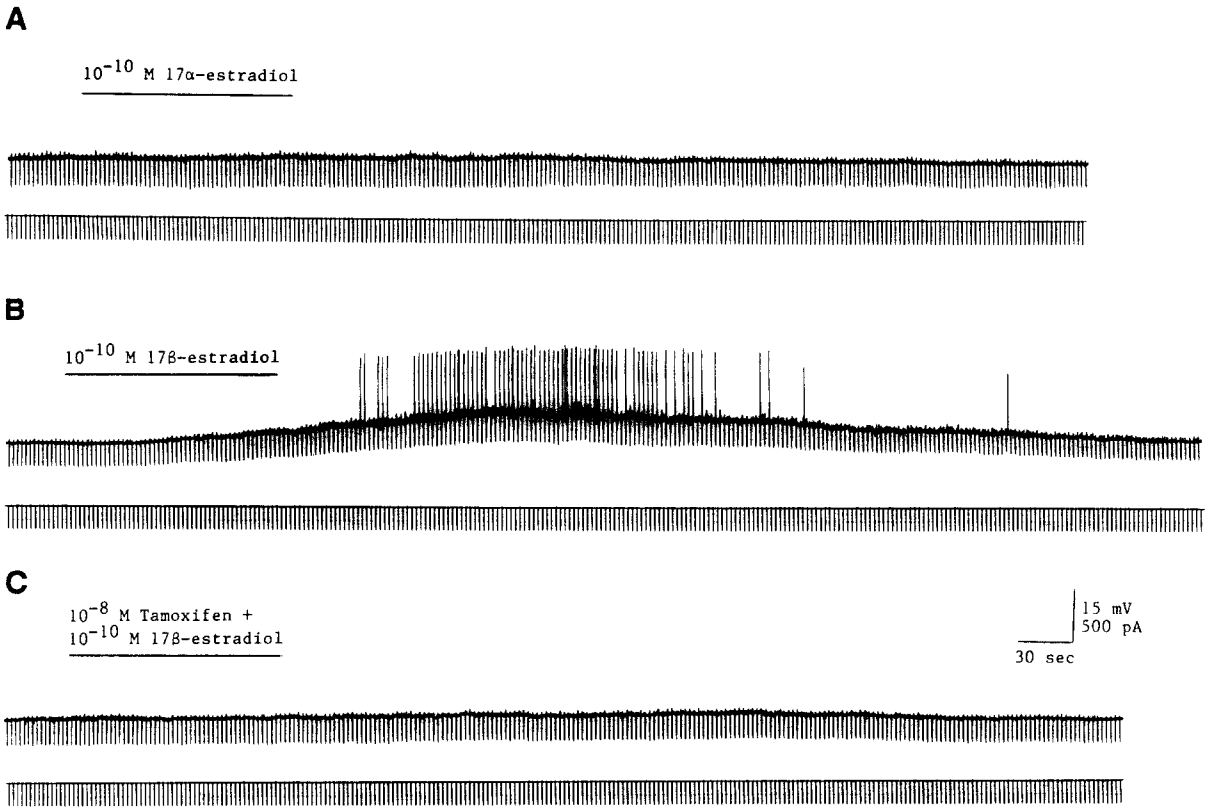

Fig. 2. Depolarization by 17β-estradiol is blocked by the estrogen antagonist, tamoxifen. All records are from the same cell. The horizontal bars denote the time of a 2-min application of 17α- or 17β-estradiol. A: 10^{-10} M 17α-estradiol has no effect on membrane potential or apparent input resistance. B: 10^{-10} M 17β-estradiol induces a reversible depolarization and increase in apparent input resistance. C: 10^{-8} M tamoxifen was superfused for 2 min prior to and also during the application of 10^{-10} M 17β-estradiol. Co-application of tamoxifen blocks the excitatory action of 17β-estradiol.

from female rats, a hyperpolarizing response to 17β-estradiol was seen. No significant changes in the accommodative properties (Fig. 1C) or the amplitude of the slow afterhyperpolarization (data not shown) of CA1 neurons were detected.

Superfusion of the 17β-estradiol in a calcium-free solution with 10 mM Mg^{2+} and 0.2 mM Cd^{2+} still produced the depolarization and increased membrane resistance. While a presynaptic contribution cannot be ruled out, this result suggests that 17β-estradiol acts directly on the CA1 pyramidal cell membrane. Two out of four neurons tested, which were depolarized by 17β-estradiol alone, showed no response to 17β-estradiol in the presence of tamoxifen (Fig. 2). As tamoxifen is an anti-estrogen which can block many of the genomic-mediated actions of estrogens[5], this suggests that the excitatory effect of 17β-estradiol is mediated by a specific estrogen receptor. This putative receptor may represent a yet unidentified membrane binding site, which shares some structural homology to the intracellular estrogen receptor. Alternatively, the 17β-estradiol may enter the neuron and bind to the classical intracellular receptor, but then have a novel, rapid action on the membrane, which does not involve genomic regulation.

Table I summarizes the response of CA1 neurons to 17β-estradiol, categorized according to sex and stage of the female estrous cycle. There was no significant difference in the percentage of neurons excited by estradiol between male (22.7%) and female (18.8%) rats, as tested by χ^2 analysis of frequency. Among the different

TABLE I

Summary of the response of CA1 neurons to 17β-estradiol according to sex and stage of the estrous cycle

The number in brackets denotes the number of rats used in the indicated animal group. For each group, the data are divided into the number (and %) of cells that responded to 17β-estradiol with depolarization (excitation), hyperpolarization (inhibition), and no effect.

Animal group	*Excitation*	*Inhibition*	*No effect*	*Total cells*
Prepubertal ♀ [6]	3 (18.8%)	1 (6.2%)	12 (75%)	16
Diestrus ♀ [7]	4 (25%)	1 (6.2%)	11 (68.8%)	16
Proestrus ♀ [7]	4 (22.2%)	0 (0%)	14 (77.8%)	18
Estrus ♀ [6]	1 (7.1%)	0 (0%)	13 (92.9%)	14
Total ♀ [26]	12 (18.8%)	2 (3.1%)	50 (78.1%)	64
♂ [9]	5 (22.7%)	0 (0%)	17 (77.3%)	22
Total [35]	17 (19.8%)	2 (2.3%)	67 (77.9%)	86

groups of female rats, estradiol-responsive cells were found in prepubertal (18.8% of cells tested) and adult females in all stages of the estrous cycle. A non-significantly, but noticeably, smaller percentage of neurons were excited by 17β-estradiol from the group of estrus females (7.1%), when compared to proestrus (22.2%) and diestrus (25%) females.

The predominant electrophysiological effect of 17β-estradiol on CA1 neurons was a depolarization, triggering of action potentials, and an increase in apparent input resistance. A number of other studies have also reported excitatory effects of estrogens on various areas of the nervous and endocrine systems. Intracellular recordings from hypothalamic[13] and pituitary cells[1] have found that 17β-estradiol induces a depolarization and increased input resistance, similar to the present study. Single unit extracellular studies have demonstrated an increase in firing rate of hypothalamic[6,14], as well as hippocampal cells[7] in response to 17β-estradiol. Furthermore, 17β-estradiol has been shown to increase the amplitude of the CA1 population spike in hippocampal slices[4,20]. In addition to the excitatory effect of 17β-estradiol, a hyperpolarizing response was also seen in two neurons in the present study. Similar results have also been reported in the ventromedial hypothalamus, where 17β-estradiol can cause either a depolarization or hyperpolarization[13]. Furthermore, these opposing responses in hypothalamic neurons appear to differ in their linkage to cyclic AMP.

In this study, the percentage of CA1 neurons responsive to 17β-estradiol was not significantly different when comparing male and female rats. This is consistent with the autoradiographic findings that male and female rats exhibit similar estradiol-binding in the hippocampus[8]. In contrast, the previous study measuring extracellular CA1 field potentials had found a more prominent effect of estradiol on male rats[20], whereas estradiol-induced hyperpolarizations in the amygdala were more common in female rats[16]. We found that a non-significantly smaller percentage of neurons were excited by 17β-estradiol from female rats in estrus, versus other stages of the estrous cycle. As endogenous estrogen levels are low during estrus following the estrogen peak of proestrus, this finding may indicate that estrogen receptors are down-regulated or desensitized during estrus.

The present results provide further evidence that estrogens exert a rapid, short-term action on neuronal excitability. The short latency of the response (<1 min) indicates that the effect does not utilize the classical genomic mechanism of steroid action. While estradiol binding sites have been demonstrated in the hippocampus[8,10,12,17], it has not yet been determined whether any of these binding sites represent membrane receptors. The demonstration of steroidal binding sites on neural membranes in other preparations[11,21], however, makes the possibility of membrane receptors for estradiol in the hippocampus likely. Progesterone metabolites have been shown to interact with the $GABA_A$ receptor in cultured hippocampal neurons[11]. Similarly, glutamate receptors have been proposed as possible target sites for estrogen[19]. Given the abundance of excitatory amino acid receptors in the hippocampus, interaction of 17β-estradiol with a glutamate-activated channel might account for the excitatory effects seen in this study. Another possible mechanism for 17β-estradiol-induced depolarization may involve a G-protein-linked receptor stimulating production of cyclic AMP[13].

Female gonadal steroids can influence sexual behavior, affect[9], and learning and memory[22], as well as release of hypothalamic releasing-factors, suggesting a prominent role of estrogens in regulating the activity of the limbic system. Estrogen binding-sites have been localized in many components of the limbic system, including the hypothalamus, septum, amygdala, and hippocampus. Furthermore, 17β-estradiol has been reported to exert rapid effects on the membrane excitability in these same limbic structures, as already discussed. While the major action of estrogen in regulating the functional output of the limbic system most likely involves the classical genomic mechanism, a rapid membrane effect of estrogens, as reported in this and other studies, may serve as an additional short-term mechanism for steroidal modulation of neural activity.

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