Cellular/Molecular

Estradiol Regulates the Slow Ca^{2+}-Activated K^{+} Current in Hippocampal Pyramidal Neurons

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The slow Ca^{2+}-activated K^{+} current (sI_{AHP}) was recorded in CA1 pyramidal neurons in hippocampal slices obtained from ovariectomized (OVX) or sham OVX (control) female rats. The sI_{AHP} was significantly larger in cells from OVX rats than in cells from control rats. Superfusion with 5–100 nM 17β-estradiol (E2) caused a progressive decrease in the sI_{AHP} in cells from OVX rats but not in cells from control rats. In slices from OVX rats injected with 10 μg of E2 24 and 48 hr before they were killed, superfusion with E2 did not modify the sI_{AHP}. In neurons from OVX rats, but not in neurons from control rats, E2 significantly increased both the number of action potentials and the burst duration generated by depolarizing pulses. The inactive isomer 17α-estradiol had no effect. The impermeant protein conjugate E2–BSA was as effective as free E2 at decreasing the sI_{AHP}. Ca^{2+} spikes were also depressed by E2 in neurons from OVX rats, but not in control rats. A decrease in the intracellular Ca^{2+} signal, correlating with the inhibition of the Ca^{2+} spike and sI_{AHP} produced by E2, was observed only in neurons from OVX rats. Our results indicate that ovariectomy increases the sI_{AHP} and depresses excitability, whereas bath application or priming with E2 decreases the sI_{AHP}, thus promoting excitability. These effects of E2 on the sI_{AHP} and excitability, which are stereospecific and presumably mediated by membrane-bound receptors, could contribute to the hormonal regulation of synaptic plasticity and epileptiform activity as well as to learning and cognitive abilities dependent on the function of hippocampal neural circuits.

Key words: **estradiol; excitability; hyperpolarizing potassium current; calcium-dependent potassium current; calcium signal; hippocampus; estrogen effects**

Introduction

Levels of circulating estrogen influence aspects of behavior that are not directly related to reproduction. Cognitive performance and learning ability in experimental animals (Fader et al., 1998; Daniel et al., 1999; Gibbs, 2000) and humans (Hampson, 1990b; Henderson et al., 1996; Asthana et al., 1999; Costa et al., 1999; Drake et al., 2000; Duka et al., 2000; Yaffe et al., 2000) vary concurrently with the reproductive cycle or with experimentally induced changes in estrogen levels. For example, administration of estrogen improves tests of spatial learning and navigation, tasks in which hippocampal neural circuits have been shown to be involved (Hampson, 1990a; Galea et al., 1995; Mead and Hampson, 1997). The precise mechanisms for these effects of estrogen are not fully understood. Reports from Moss' laboratory (Wong and Moss, 1991, 1992, 1994; Gu and Moss, 1998; Moss and Gu, 1999) suggest that the hormone regulates synaptic transmission. They described short-term and long-term effects of estradiol, all conducive to facilitated excitatory input, mediated at least in part by "fast" actions through putative membrane receptors. Moreover, estrogen can enhance long-term potentiation (LTP) (Córdoba Montoya and Carrer, 1997; Foy et al., 1999; Good et al., 1999), which could be a consequence of increased synaptic effectiveness resulting from stimulated dendritic spinogenesis, increased glutamate sensitivity, and/or a new balance of excitatory and inhibitory input (Rudick and Woolley, 2001) occurring in the hippocampus (for review, see McEwen et al., 2001).

In addition to improving synaptic transmission, estradiol could act to modulate the membrane mechanisms that control neuronal excitability and repetitive firing. In hippocampal pyramidal cells, action potentials are followed by a multicomponent afterhyperpolarization (AHP) comprising a fast AHP, a medium AHP, and a slow AHP (sAHP) (for review, see Storm, 1990; Sah and Davies, 2000). These AHPs act as a negative feedback regulating excitability and spike frequency adaptation and could be regulated by estrogen.

The following studies were designed to investigate whether estrogen could regulate the slow Ca^{2+}-activated K^{+} current (sI_{AHP}) that mediates the sAHP. We recorded the sI_{AHP} of CA1 pyramidal neurons in hippocampal slices obtained from animals that had been ovariectomized (OVX) 4–5 weeks in advance and compared the responses with those of sham OVX rats. We show that neuronal excitability was markedly reduced, and that both the amplitude and area of the sI_{AHP} were notably larger in OVX rats than in control rats. Furthermore, superfusion with estradiol sig-

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Table 1. Membrane properties of CA1 neurons from OVX and control rats

				Action potential		
	Resting potential (mV)	Input resistance (MΩ)	Time constant (msec)	Duration (msec)	Adaptation (number of APs)	Burst duration (msec)
Control	61.6 ± 8.0 (13)	99 ± 8 (11)	9.5 ± 1.9 (17)	3.9 ± 0.1 (11)	6.0 ± 0.6 (14)	290 ± 58 (14)
OVX	57.2 ± 5.0 (20)	92 ± 4 (24)	7.8 ± 1.5 (33)	4.1 ± 0.1 (31)	4.0 ± 0.4* (20)	148 ± 34** (20)

Data are mean ± SEM. Number of cells are in parentheses.

*$p < 0.05$; **$p < 0.01$ versus control (Student's *t* test).

nificantly reduced the sI_{AHP} and increased neuronal excitability in slices from ovariectomized animals. However, the sI_{AHP} was unaffected by estradiol in animals previously exposed to estrogen (i.e., in both control and estradiol-primed ovariectomized rats). Furthermore, estradiol reduced the amplitude of depolarization-evoked Ca^{2+} spikes in slices from OVX rats. Finally, superfusion of estradiol in slices from ovariectomized rats but not from control rats reduced the depolarization-evoked intracellular Ca^{2+} elevation that correlated with the estradiol-mediated sI_{AHP} reduction. We conclude that estrogen regulates the sI_{AHP} and neuronal excitability, and that these effects are mediated by specific membrane receptors that control Ca^{2+} influx, thus modulating the intracellular Ca^{2+} signal.

Materials and Methods

OVX and sham OVX (control) female Wistar rats were used. Surgery was performed under cold anesthesia 2 d after birth (Yi and Barr, 1996). Pups were ovariectomized through a bilateral laparotomy; the skin was sutured and the incision was covered with the acrylic polymer Nobecutan (Inibsa Laboratorios, Barcelona, Spain) before they were returned to their mothers. In sham OVX animals, the ovaries were exposed and the skin was sutured. Animals were decapitated at 35–60 d of age, and brains were rapidly removed and submerged in ice-cold artificial CSF (ACSF). All experiments in this study conformed to international guidelines on the ethical use of animals, and every effort was made to minimize the suffering and number of animals used.

Transverse 300 μm slices of the hippocampus were cut with a vibratome (Pelco 101 Series 1000; Pelco, St. Louis, MO) and preincubated for 1 hr at room temperature in ACSF continuously bubbled with carbogen (95% O_2, 5% CO_2). The incubation ACSF contained (in mM): 124 NaCl, 2.6 KCl, 1.25 KH_2PO_4, 2 Mg_2SO_4, 26 $NaHCO_3$, 2 $CaCl_2$, and 10 glucose, pH 7.4. Slices were transferred to a 2 ml chamber fixed to an inverted microscope stage (Diaphot TMD; Nikon, Tokyo, Japan) and superfused continuously at 1 ml/min at room temperature (21–23°C) with ACSF containing 50 μM picrotoxin. Recordings in the whole-cell configuration of the "blind" patch-clamp technique used 4–7 MΩ pipettes filled with (in mM): 150 $KMeSO_4$ (ICN Pharmaceuticals, Costa Mesa, CA), 10 HEPES, and 4 ATP-Na. Pipettes were connected to an Axoclamp-2A amplifier (Axon Instruments, Foster City, CA), and recordings were in the bridge-electrode or single-electrode current-clamp mode and single-electrode voltage-clamp mode. The series resistance was compensated to 60–80%, and capacitances were neutralized.

Data were low-pass filtered at 1 kHz (VBF4; Kemo, Beckenham, UK) and sampled at rates of >2 kHz with a 486 personal computer and a TL-1/DMA interface board (Axon Instruments). pClamp software (Axon Instruments) was used for experimental control, data acquisition, and analysis. Recording pipettes were positioned using a micromanipulator, and the CA1 soma layer was visualized directly. Pyramidal cells were recognized by their characteristic responses to depolarizing current pulses under current clamp (Borde et al., 1995, 2000). Only one neuron was studied per slice.

Experiments in both current-clamp or voltage-clamp modes were started subsequent to stabilization period of ~5 min after access to the intracellular compartment. Two experimental protocols were used in the current-clamp mode. To evaluate passive membrane properties (Table 1; resting potential, membrane input resistance, and time constant), 10 successive 200 msec depolarizing pulses starting from 0.3 nA, and increasing in 0.08 nA steps, were delivered at rates of 1 sec^{-1} in current-clamp mode. Additionally, changes in cell excitability were evaluated in current-clamp mode by calculating the mean number of action potentials (APs) and the duration of bursts evoked by single 2 sec, 0.5 nA depolarizing current pulses delivered every 5–10 min while holding the membrane potential (V_m) at −60 mV (Table 1; action potential duration, adaptation, and burst duration).

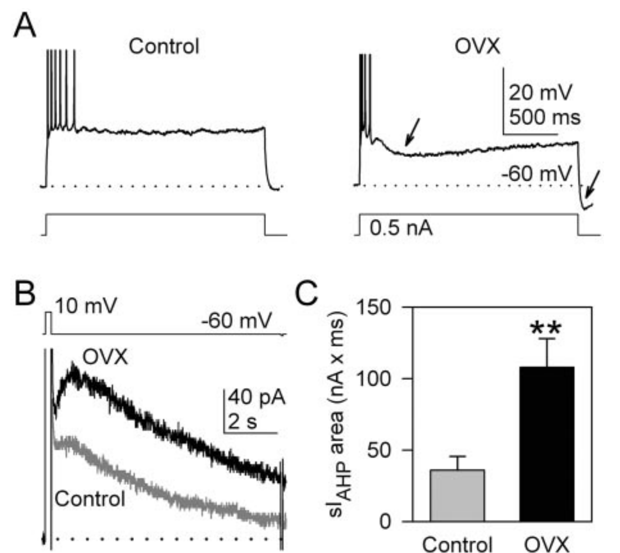

Figure 1. OVX rats showed larger sI_{AHP} and AP firing adaptation than control rats. *A*, Representative current-clamp recordings of neurons from control and OVX rats. Note the difference in AP adaptation between neurons from control and OVX rats, and the larger AHP in OVX rats (arrows). Membrane potential was −60 mV (dotted lines). Action potentials are truncated for illustration purposes. *B*, Superimposed sI_{AHP} evoked by 200 msec depolarizing pulses (top trace) generated in CA1 pyramidal neurons from an OVX (black trace) and a control (gray trace) rat. *C*, Mean area of sI_{AHP} recorded in neurons from control ($n = 28$) and OVX ($n = 41$) rats. **Significant differences were estimated with the Student's *t* test at $p < 0.005$.

To study the sI_{AHP}, membrane currents were evoked by 200 msec depolarizing voltage commands, from a −60 or −50 mV holding potential (V_h) to +10 mV. To avoid possible interference between the responses, depolarizing pulses were delivered every 5–10 min. The sI_{AHP} magnitude was quantified from the area under the current trace, measured 200 msec after the end of the pulse [when the amplitude of the medium I_{AHP} (mI_{AHP}) was negligible] (Martín et al., 2001). The mI_{AHP} amplitude was measured ~50 msec after the end of the pulse. To simultaneously monitor the access and membrane resistance and capacitance, the voltage-clamp protocol included a 70 msec, 5 mV hyperpolarizing pulse at the end of the recording 8 sec epoch (Fig. 1*A*). Preliminary experiments showed that sI_{AHP} was relatively small immediately after breaking the cell membrane but increased rapidly to stabilize in 10–15 min. For this reason, after establishing the whole-cell configuration, control recordings were obtained over ~20 min. After obtaining control recordings (usually three to six), superfusion was started with ACSF containing 5, 10, or 100 nM 17β-estradiol (E2). Superfusion with E2 was maintained for 40 min and then replaced with normal ACSF. The E2 was dissolved in absolute ethanol and added to ACSF; final ethanol concentrations never exceeded 0.01%. E2 conjugated to bovine serum albumin

[17β-estradiol -6-(*O*-carboximetil)oxima (E2–BSA)] was made up as a concentrated (100 μM) stock solution in ACSF, aliquoted, and frozen (−20°C) until use at a final concentration of 100 nM in ACSF. The concentration of BSA was matched in the control ACSF in experiments testing the effect of E2–BSA. Identical experimental protocols were used when studying the effect of 17α-estradiol.

Ca^{2+} spikes were recorded in current-clamp mode in the presence of 0.5 or 1 μM tetrodotoxin (TTX; Tocris Cookson, Bristol, UK) and 5 mM tetraethylammonium (TEA). Ca^{2+} spikes were generated by 0.5 sec, 0.3 nA depolarizing current pulses while holding the V_m at −60 mV.

Measurement of intracellular Ca^{2+} variations was performed on hippocampal slices obtained as described above. Pyramidal cells in the CA1 region were visualized under an BX50WI microscope (Olympus Optical, Tokyo, Japan) equipped with infrared and differential interference contrast imaging devices, and with a 40× water immersion objective. Patch pipettes were filled with the standard internal solution containing 10 μM Fluo-3 (Molecular Probes, Eugene, OR). Cells were illuminated with a xenon lamp at 490 nM using a monochromator Polychrome II (T.I.L.L. Photonics, Planegg, Germany). Fluorescence intensity was collected by a photomultiplier tube (model R928; Hamamatsu Photonic, Bridgewater, NJ) from a variable rectangular window (side: 25–50 μm) that included the apical dendrite and most of the neuronal soma. The fluorescence signal collected was integrated using the photometry system (T.I.L.L. Photonics) (Martín et al., 2001). Intracellular calcium variations were recorded under voltage-clamp conditions. After obtaining at least two control records, slices were superfused for 40 min with 100 nM E2 or 100 nM 17α-estradiol and records were obtained every 10 min. The voltage-clamp command was as described previously. Data are expressed as means ± SEM. Statistically significant differences were estimated with the Student's *t* test, unless indicated otherwise.

Drugs were obtained from Sigma (St. Louis, MO) unless specified otherwise.

Results

No significant differences were found in resting V_m (input), membrane resistance, membrane capacitance, or duration of evoked APs in a representative sample of CA1 pyramidal neurons recorded from OVX ($n = 41$) and control ($n = 28$) rats (Table 1). Although all neurons tested from OVX or control rats showed marked spike frequency adaptation during the 2 sec depolarizing current pulses of 0.5 nA, the mean number of APs was smaller and the burst duration was briefer in OVX rats compared with control rats (Table 1; $p < 0.05$ and $p < 0.01$, respectively), indicating a decreased neuronal excitability in OVX rats (Fig. 1*A*).

Effects of E2 on the sI_{AHP}

In neurons from control rats, the sI_{AHP} peaked at 452 ± 35 msec ($n = 28$) after termination of the depolarizing pulse, whereas in neurons from OVX rats, the sI_{AHP} peaked at 613 ± 77 msec ($n = 41$; $p = 0.03$). Both peak amplitude (OVX, 46 ± 5 pA; control, 24 ± 5 pA; $p = 0.02$) and area under the curve (OVX, 108 ± 20 nA × msec, $n = 41$; control, 36 ± 9 nA × msec, $n = 28$; $p = 0.005$) of the sI_{AHP} were significantly larger in cells from OVX rats than in cells from control rats (Fig. 1*B,C*). The decay of the sI_{AHP} could be fitted to a single exponential function, and no significant differences were observed in the time constant of the sI_{AHP} decay measured in control versus OVX rats (2745 ± 271 and 3105 ± 279 msec, respectively).

Superfusion with 5–100 nM E2 caused a progressive decrease in the sI_{AHP} in cells recorded in slices from OVX rats. This effect became significant after 20 min of superfusion with E2 (repeated-measures ANOVA; df = 2, 28; $F = 7.177$; $p = 0.003$) and stabilized thereafter up to the end of the superfusion with E2 (56 ± 7% of control values; $n = 20$) (Fig. 2). When superfusion with normal ACSF was resumed after E2, the sI_{AHP} remained at depressed values. Indeed, in nine cells in which stable recordings could be

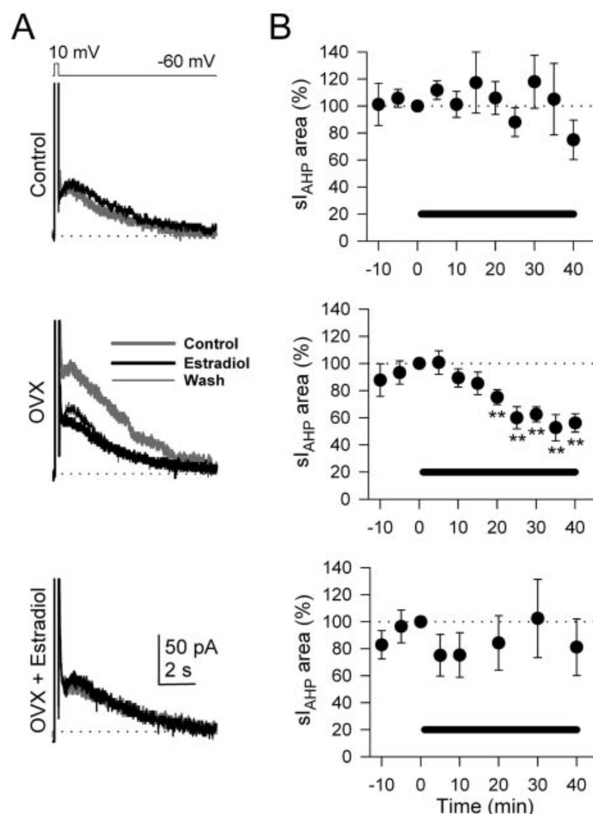

Figure 2. Effect of estradiol on sI_{AHP}. *A*, Representative traces obtained before (control, gray thick traces), 20 min after superfusing with 100 nM E2 (estradiol, black traces), and 20 min after superfusing with normal ACSF (wash, black thin traces) in neurons from control rats, OVX rats, and OVX rats primed with E2 (10 μg) 24 – 48 hr before they were killed (top, middle, and bottom panels, respectively). *B*, Relative sI_{AHP} area obtained in neurons from control rats ($n = 15$), OVX rats ($n = 30$), and OVX rats primed with E2 ($n = 6$) (top, middle, and bottom panels, respectively) before and during superfusion with 100 nM E2 (black horizontal bar). Data are presented as a percentage of the control sI_{AHP} recorded before starting superfusion with E2. **Significant differences were estimated with ANOVA at $p < 0.003$. The dotted horizontal lines in *A* and *B* correspond to baseline and control values, respectively.

obtained after 40 min of superfusion with normal ACSF, the sI_{AHP} was 58 ± 11% of control values (data not shown).

In slices obtained from control rats, superfusion with 100 nM E2 for 40 min produced no significant decrease in the sI_{AHP} (75 ± 15% from control values; $n = 12$) (Fig. 2). To determine whether priming with estrogen would change the response observed after superfusion with E2, OVX rats were injected with 10 μg of E2 24 and 48 hr before they were killed. In slices obtained from these rats, the mean sI_{AHP} area was not statistically different from control rats (47 ± 10 nA × msec, $n = 6$; 36 ± 9 nA × msec, $n = 28$, respectively), and superfusion with E2 did not significantly modify the sI_{AHP} (Fig. 2) (81 ± 21% from controls; $n = 6$). Therefore, E2 regulates the sI_{AHP} of OVX rats without modifying the sI_{AHP} in rats that have been exposed to estrogen previously (i.e., both control and E2-primed OVX rats).

Likewise, the mI_{AHP} amplitude was also significantly higher in neurons from OVX rats (83 ± 12 pA; $n = 41$) than from control rats (39 ± 11 pA; $n = 28$; $p = 0.01$) (Figs. 1*B*, 2*A*). Furthermore, E2 superfusion reduced the amplitude of the mI_{AHP} in neurons from OVX rats (60 ± 7% from control values; $n = 20$; $p = 0.001$; sign test), but not in neurons from control rats (84 ± 12% from control values; $n = 12$), indicating similar effects of estrogen on both sI_{AHP} and mI_{AHP} (see Figs. 2*A*, 3, 6*A,C*). The present study

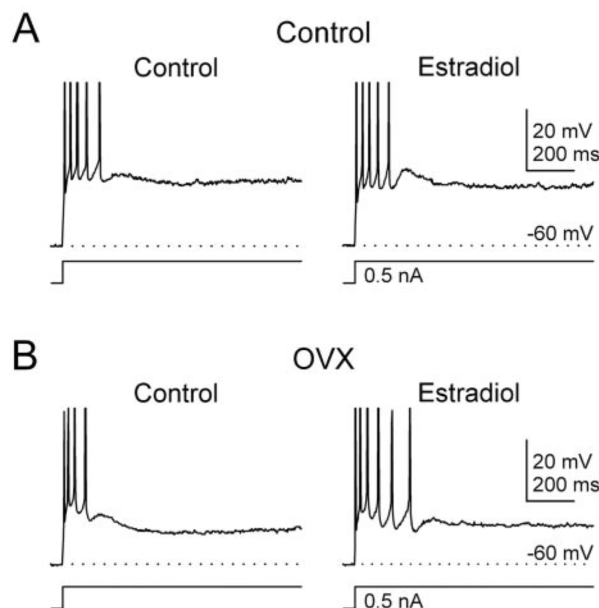

Figure 3. Effect of E2 on the excitability of neurons. *A, B*, Current-clamp responses evoked by 0.5 nA depolarizing steps showing the effect of E2 on the AP adaptation in control and OVX rats, respectively, before (control) and after 20 min of perfusion with 100 nM estradiol. Membrane potential was −60 mV (dotted lines). Action potentials are truncated for illustration purposes.

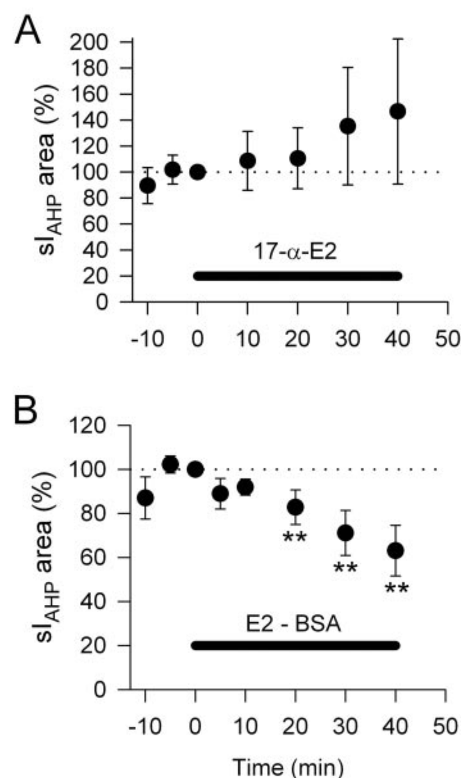

Figure 4. Effects of the inactive isomer 17α-estradiol and the impermeable E2–BSA conjugate on the area of the sI_{AHP} in ovariectomized rats. *A*, The sI_{AHP} area was not significantly modified by superfusion with the inactive isomer 17α-estradiol (100 nM) ($n = 4$). *B*, The impermeable E2–BSA conjugate (100 nM) reduced the area of the sI_{AHP} ($n = 5$). Results are expressed as a percentage of the sI_{AHP} area recorded before hormone superfusion. Horizontal bars indicate the time of hormone superfusion. **Significant differences were estimated with ANOVA at $p < 0.005$. The dotted horizontal lines correspond to control values.

focused on the sI_{AHP}, and changes in the mI_{AHP} were not considered further.

Effects of E2 on neuronal excitability

The sI_{AHP} is a Ca^{2+}-dependent K^+ current that plays a key role in the control of neuronal excitability (Storm, 1990; Borde et al., 1995; Sah and Davies, 2000). We therefore investigated the consequences of E2-mediated regulation of the sI_{AHP} on the electrical excitability of CA1 pyramidal neurons.

During current-clamp recordings in neurons from OVX rats, superfusion with E2 increased both the number of APs (from 3.9 ± 0.4 to 5.7 ± 1.0; $n = 20$; $p = 0.003$; sign test) and the burst duration (from 148 ± 34 to 242 ± 60 msec; $n = 20$; $p = 0.001$; sign test) during depolarizing pulses presented at the same V_m (Fig. 3). In neurons from control rats, superfusion with E2 had no effect on the number of APs (6.2 ± 0.6 and 5.9 ± 0.6 before and after 40 min of E2 superfusion, respectively; $n = 14$) or burst duration (290 ± 58 msec in controls and 310 ± 75 msec in the presence of E2; $n = 14$). As described above, in control rats, the number of APs and the burst duration were larger than in OVX rats, as expected from the different amplitudes of the sI_{AHP}.

No significant changes in the holding current or input resistance were found after 40 min of E2 perfusion in either control (108 ± 5 and 102 ± 2%, respectively, from control values; $n = 12$) or OVX rats (110 ± 5 and 98 ± 1%, respectively, from control values; $n = 20$). These results indicate that E2 can effectively regulate the excitability of neurons through the modulation of the sI_{AHP}.

E2 acts via specific membrane receptors

To investigate whether the inhibitory effect of E2 on the sI_{AHP} was mediated through a specific receptor, the effect of superfusion with the inactive isomer 17α-estradiol (100 nM) was tested. No significant change in the sI_{AHP} was observed after 17α-estradiol treatment ($n = 4$) (Fig. 4*A*).

To determine whether penetration of E2 through the cell membrane was necessary for the steroid to be effective, we analyzed the actions of the membrane impermeant protein conjugate E2–BSA on the sI_{AHP} in neurons from OVX rats. As shown in Figure 4*B*, the sI_{AHP} area was decreased after superfusion with 100 nM E2–BSA ($n = 5$; ANOVA; df = 5, 16; $F = 5.563$; $p = 0.005$). Indeed, the sI_{AHP} area was reduced 63 ± 11% ($n = 5$) from control values by 40 min of superfusion with E2–BSA, which was not significantly different from the reduction induced by E2 (Fig. 2) (56 ± 7%; $n = 20$), indicating that E2–BSA was as effective as free E2 at decreasing the sI_{AHP}. These results indicate that the effect of E2 on the sI_{AHP} is stereospecific and suggest that the receptor mediating this effect is located at the membrane of CA1 pyramidal neurons.

E2 reduces depolarization-evoked Ca^{2+} spikes

We also studied the possible cellular mechanisms involved in the depression of the sI_{AHP}. Because the sI_{AHP} is a Ca^{2+}-dependent current, we investigated the possibility that the reduced sI_{AHP} may be caused by E2 modulation of Ca^{2+} influx, which was evaluated by the amplitude and area of Ca^{2+} spikes. In current-clamp mode, after superfusing the slice with TTX and TEA, depolarizing current pulses evoked spikes of >100 msec duration and 80–100 mV amplitude (Fig. 5*A*). These spikes were stable for at least 60 min and were suppressed by perfusion with 100 μM Cd^{2+} ($n = 2$; data not shown). Perfusion with 100 nM E2 caused a significant decrease in amplitude and area ($n = 5$; ANOVA; df = 4, 16; $F = 6.82$; $p = 0.002$) of the Ca^{2+} spikes (Fig. 5*A,B*).

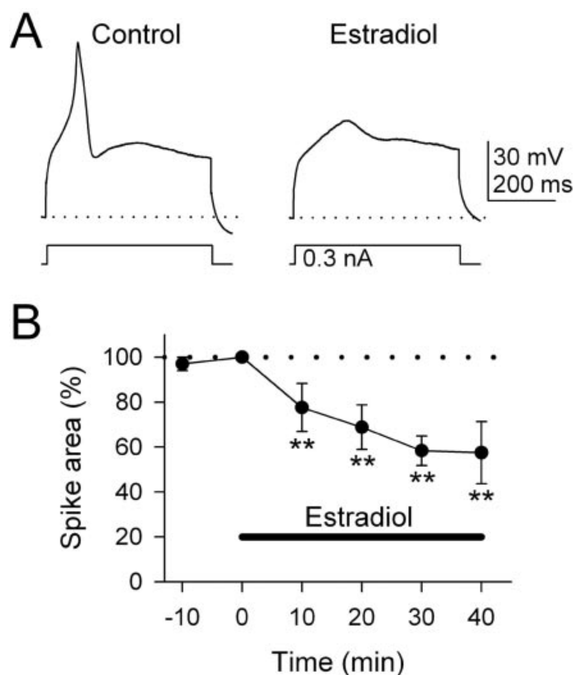

Figure 5. Effects of estradiol on depolarization-activated Ca^{2+} spikes recorded from pyramidal neurons in slices obtained from ovariectomized rats. *A*, Current-clamp responses to 0.3 nA depolarizing pulses in the presence of 0.5 μM TTX and 5 mM TEA (control) and after 40 min of superfusion with 100 nM estradiol. Membrane potential was −60 mV. *B*, Relative Ca^{2+} spike area before and during superfusion of 100 nM estradiol (horizontal bar) ($n = 5$). Data are expressed as a percentage of the Ca^{2+} spike area recorded before hormone superfusion was initiated. The Ca^{2+} spike area was quantified from the area enclosed by the voltage trace (between 100 msec before and after the peak value) over the depolarization-evoked steady-state potential. **Significant differences were estimated with ANOVA at $p < 0.002$. The dotted horiztonal lines in *A* and *B* correspond to baseline and control values, respectively.

E2 reduces the depolarization-evoked intracellular Ca^{2+} signal

We also studied the possibility that reduction of Ca^{2+} influx may affect the intracellular Ca^{2+} signal paralleling the modifications of the sI_{AHP}. As expected, membrane depolarization evoked a rapid increase in the Ca^{2+} signal that slowly decayed during the sI_{AHP} deactivation (Martín et al., 2001). As described above, although the sI_{AHP} in control rats was not significantly affected by superfusion with 100 nM E2, in OVX rats, the sI_{AHP} was decreased after E2 treatment (Fig. 6*A*,*C*). A consistent decrease in the Ca^{2+} signal, which paralleled the inhibition of the sI_{AHP} produced by E2, was observed in cells ($n = 8$) from OVX rats (Fig. 6*D*), whereas no significant decrease in the Ca^{2+} signal was observed in cells ($n = 4$) from control rats (Fig. 6*B*).

Statistical analysis (by ANOVA) indicated a significant treatment (control vs OVX) effect (df = 1, 10; $F = 6.28$; $p = 0.03$) and a significant interaction between time and treatment (df = 4, 40; $F = 4.91$; $p = 0.003$). *Post hoc* analysis indicated significant differences (control vs OVX; $p < 0.001$) starting at 20 min (Fig. 6*E*). These results imply that E2 inhibited the depolarization-evoked Ca^{2+} signal in OVX rats. Furthermore, the E2-induced inhibition of the Ca^{2+} signal and the sI_{AHP} showed a strong linear relationship ($r = 0.99$) (Fig. 6*F*, filled symbols), suggesting that the E2-evoked inhibition of the Ca^{2+} signal was responsible for the modulation of the sI_{AHP} by E2. Moreover, the mean effects of E2 on the Ca^{2+} signal and on the Ca^{2+} spike area (measured in current-clamp conditions in different cells) (Fig. 5) could also be accurately fitted to a linear regression ($r = 0.97$) (Fig. 6*F*, open

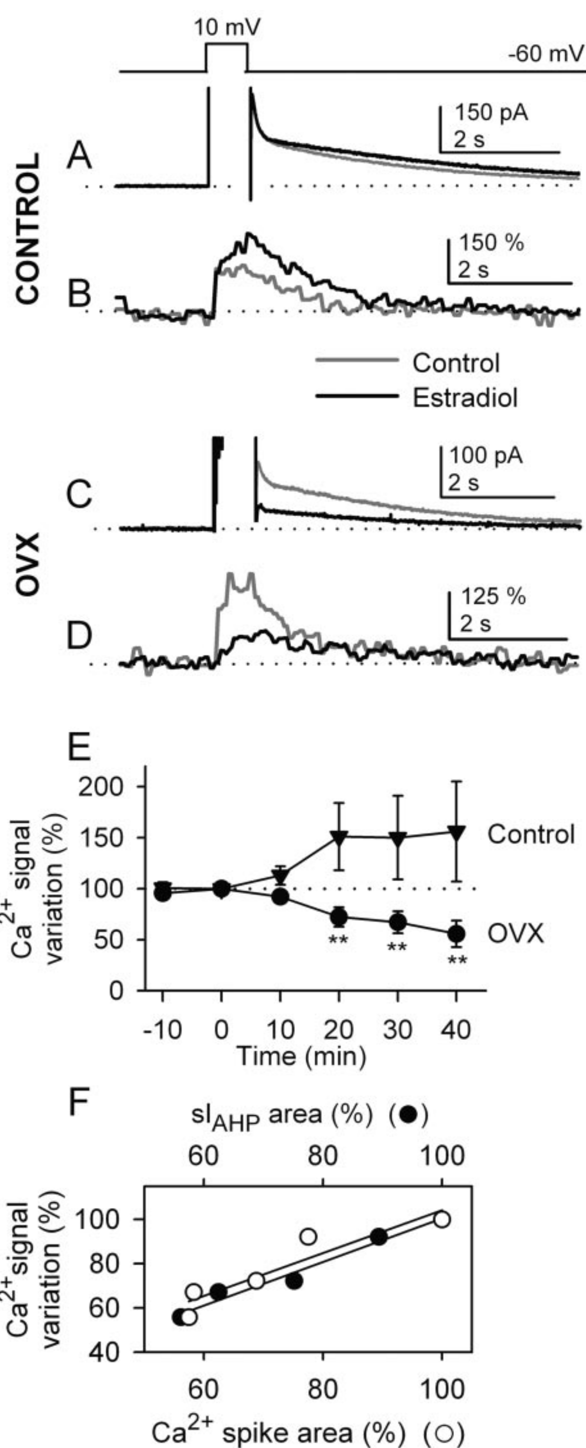

Figure 6. Effects of estradiol on the sI_{AHP} and the intracellular Ca^{2+} signal. *A–D*, Simultaneously recorded sI_{AHP} (*A, C*) and intracellular Ca^{2+} signal (*B, D*) evoked by depolarizing pulses in pyramidal neurons in slices obtained from control (*A, B*) and OVX (*C, D*) rats. Control recordings obtained before superfusion with E2 are drawn in gray, and recordings obtained 40 min after superfusion with E2 are drawn in black. *E*, Effects of superfusion with E2 on Ca^{2+} signal in neurons of slices obtained from control ($n = 4$) (triangles) and OVX ($n = 8$) (circles) rats. **Significant differences were estimated with ANOVA at $p < 0.002$. *F*, Linear regression of the mean changes in intracellular Ca^{2+} variation versus mean changes in the area of the Ca^{2+} spike (open symbols) and the sI_{AHP} area (filled symbols) at 0–40 min of superfusion with E2. Intracellular Ca^{2+} variation and the sI_{AHP} area were simultaneously recorded from the same cells, whereas values of the Ca^{2+} spike area were obtained from different cells. The dotted horizontal lines in *A–E* corespond to baseline and control values, respectively.

symbols), suggesting that the E2-induced reduction of the depolarization-evoked Ca^{2+} spikes was the limiting factor for the E2-mediated inhibition of the intracellular Ca^{2+} variations.

Together, these results indicate that E2 regulates neuronal excitability by modulating the sI_{AHP} via regulation of the influx of Ca^{2+} through voltage-gated Ca^{2+} channels.

Discussion

The results described above indicate that manipulation of circulating E2 levels affects the sI_{AHP} of CA1 pyramidal neurons. First, CA1 pyramidal neurons in OVX rats show a notably larger sI_{AHP} than cells from normal rats. This difference is also reflected in the excitability of neurons, because a greater number of APs and longer bursts were evoked by membrane depolarization in neurons from control rats. Second, CA1 pyramidal neurons respond to E2 in quite a different manner when applied in control or OVX rats. Superfusion with E2 in neurons obtained from control rats had no effect on the sI_{AHP}, whereas in OVX rats, E2 produced a significant and sustained reduction of the sI_{AHP}, accompanied by increased excitability. These effects were stereospecific, because 17α-estradiol was ineffective.

Priming OVX rats with E2 before they were killed prevented the effect of superfused E2. The action of superfused E2 on the sI_{AHP} appears to be mediated by membrane-bound receptors, because an impermeant conjugate of the steroid was as effective as the free form. The amplitude and area of Ca^{2+} spikes evoked under TTX were reduced by E2 superfusion in slices from OVX rats, suggesting a modulation of Ca^{2+} influx by E2. In addition, a reduction of the intracellular Ca^{2+} signal evoked by membrane depolarization correlated with the reduction of the sI_{AHP} produced by estrogen superfusion, supporting the hypothesis that the main reason for the smaller sI_{AHP} is a reduction of the influx of Ca^{2+}, which leads to a reduced activation of Ca^{2+}-dependent K^+ channels.

Many studies have investigated the effects of gonadal steroids on hippocampal function. Previous reports focused on the transynaptic and receptor-mediated effects, showing that E2 facilitates excitatory responses of CA1 pyramids (Wong and Moss, 1992, 1994; Woolley et al., 1997), and that pyramidal neurons from adult OVX animals primed with E2 fire repetitively in response to stimulation (Wong and Moss, 1992), thus probably contributing to the increased bursting activity that characterizes cognitive-related hippocampal activity (Larson and Lynch, 1986; Huerta and Lisman, 1993).

The level of "spontaneous" neuronal activity and synaptic responses is considerably affected by the magnitude of the sI_{AHP}, because by hyperpolarizing and shunting the membrane after a series of APs, the sI_{AHP} greatly reduces excitability and synaptic efficacy (Borde et al., 1999). As this work was in preparation, Kumar and Foster (2002) reported that E2 reduced AHP in OVX rats; this result is hereby confirmed and directly explained by the decreased sI_{AHP} observed in our experiments. The increase in the sI_{AHP} found in OVX animals and its reduction by E2 can help explain the well known depressing effects of ovariectomy on many CNS functions and the compensating effects of E2 treatment. For example, the increase in the sI_{AHP} may explain the difficulty in inducing LTP in OVX animals, because reduced excitability will make the needed coincidence of presynaptic and postsynaptic activity to meet the Hebbian rule less likely (Brown and Chattarji, 1995). Furthermore, treatment with estrogen has been shown to increase synaptic plasticity, facilitating the induction of and potentiating the LTP (Córdoba Montoya and Carrer, 1997; Foy et al., 1999). We found that superfusion with E2 reduced the sI_{AHP} in cells from OVX rats, increasing excitability and decreasing spike frequency adaptation. The effects of ovariectomy and of E2 restitution on synaptic plasticity could be partially explained by estrogen modulation of the sI_{AHP}.

The sI_{AHP} is a K^+ conductance activated by an increase in intracellular free Ca^{2+}, mediated at least in part through L-type Ca^{2+} channels (Sah, 1996; Borde et al., 1999) and Ca^{2+}-dependent Ca^{2+}-release from intracellular stores (Tanabe et al., 1998; Borde et al., 1999). There are then several mechanisms that could be affected by the lack of estrogen to produce the observed increase in sI_{AHP} in OVX rats and its reduction by superfused E2. The lack of estrogen could increase K^+ conductances, and superfusion with E2 could decrease those conductances. As far as we know, the pharmacological and single-channel studies necessary to determine whether E2 can directly affect K^+ channel characteristics in the hippocampus have not been conducted.

We show an E2-mediated modulation of the Ca^{2+} signal, and that this modulation correlates with the E2-mediated regulation of the Ca^{2+} spike and the sI_{AHP}. The steroid could control Ca^{2+} permeability of the membrane and/or Ca^{2+} release from intracellular stores, because both sources contribute to activation of K^+ channels (Tanabe et al., 1998; Borde et al., 1999; Shah and Haylett, 2000). Estrogen receptor-deficient mice showed increased expression of the cardiac L-type calcium channel (Johnson et al., 1997), and a similar augmented expression in CA1 pyramidal neurons of OVX rats could explain the observed increase in sI_{AHP}. As a matter of fact, we obtained evidence that this may be the case, because superfusion of slices from OVX rats with E2 decreased Ca^{2+} influx. Although a direct regulation of the intracellular Ca^{2+}-release mechanisms and sI_{AHP} channels by E2 cannot be totally excluded, the strong correlation between the E2-induced changes in Ca^{2+} signal, the sI_{AHP}, and the Ca^{2+} spike supports the possibility that the regulation of Ca^{2+} influx through voltage-gated Ca^{2+} channels by E2 is the limiting factor responsible for the observed effects. This interpretation is also supported by the similar modulation observed in the sI_{AHP} and mI_{AHP}. However, additional detailed analyses are required to identify the E2-sensitive mechanism responsible for the Ca^{2+} decrease, and whether the correlation between the modulation of the Ca^{2+} signal and sI_{AHP} reflects a cause–effect link.

We have obtained evidence that the reduction of the sI_{AHP} is mediated by E2 receptors of the "membrane" type, because an albumin-conjugated membrane-impermeable E2 construct had the same effect as permeable estrogen. Evidence has been obtained suggesting that these rapid effects are mediated by a membrane-bound estrogen receptor (Gu and Moss, 1998), possibly of the α-type (Razandi et al., 1999). The exact meaning of the "membrane effects" may have to be reappraised, considering recent evidence for a new pathway of receptor-mediated endocytosis of membrane-bound E2–BSA (Moats and Ramirez, 2000).

Numerous clinical (Newmark and Penry, 1980) and experimental (Woolley, 1999) studies have demonstrated that E2 can facilitate seizure activity. Apart from the demonstrated effects of E2 on synaptic function in CA1 neurons (Woolley and Schwartzkroin, 1998), because the synaptic regulation of the sI_{AHP} in those cells has been proposed to be involved in epileptogenesis (Martín et al., 2001), the modulation of the sI_{AHP} by E2 could contribute to the initiation and/or maintenance of seizures by predisposing hippocampal circuitry to epileptiform activity. Our results add the possibility of intrinsic cellular ionic mechanisms controlling neuronal excitability to help explain systemic changes known to take place in the CNS as a consequence of variations in the circulating levels of E2.

In conclusion, our results indicate that ovarian secretions regulate the sI_{AHP}, ovariectomy increases the sI_{AHP}, and bath application or priming with E2 decreases the sI_{AHP}. E2 regulates the sI_{AHP} by controlling Ca^{2+} influx, but the precise mechanism of this modulation remains to be studied. This regulation would be a contributing factor to the "excitatory" effects of estrogen and the regulation of synaptic plasticity. The physiological and behavioral consequences of this contribution should be of considerable interest, particularly because the hippocampus plays a key role in learning and memory related to the spatial abilities of adult organisms.

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