Estradiol acutely suppresses inhibition in the hippocampus through a sex-specific endocannabinoid and mGluR dependent mechanism

Guang Zhe Huang and Catherine S. Woolley

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Animals and tissue preparation

All animal procedures were performed in accordance with the National Institutes of Health *Guide to the Care and Use of Laboratory Animals* and were approved by the Northwestern University Animal Care and Use Committee. Most experiments were done with female Sprague Dawley rats, 47-57 days old. One set of experiments was done with male Sprague Dawley rats, also 47-57 days old. All rats were housed on a 12 hr light/dark cycle with *ad libitum* access to water and phytoestrogen-free food. Females were bilaterally ovariectomized under ketamine (85 mg/kg)/xylazine (13 mg/kg) anesthesia and used for recording 3-6 days after surgery. Males were gonadally intact or anesthetized as above, castrated and used for recording 3-6 days following surgery. On the day of recording, each rat was deeply anesthetized with Euthasol (100–125 mg/kg) and transcardially perfused with ice-cold oxygenated (95% O_2/5% CO_2) sucrose artificial cerebrospinal fluid (sACSF) containing (in mM): 75 sucrose, 75 NaCl, 2 KCl, 25 $NaHCO_3$, 1.25 $NaH2PO_4$, 2.4 Na pyruvate, 1.3 ascorbic acid, 3 $MgCl_2$, 0.5 $CaCl_2$, 15 dextrose, pH 7.4. The brain was quickly removed, cooled in ice-cold oxygenated sACSF, and then hippocampal slices (300 µm) were cut with a vibratome (VT1000S; Leica). Slices were incubated for 30-45 min at 34-35°C in oxygenated regular ACSF containing (in mM): 126 NaCl, 3 KCl, 26 $NaHCO_3$, 1.25 $NaH2PO_4$, 1 $MgCl_2$, 2 $CaCl_2$, 10 dextrose, pH 7.5, and then maintained in regular ACSF at room temperature (20-22°C) until recording.

Electrophysiological recording

Each slice was transferred to a submersion chamber mounted on a *Zeiss* Axio Examiner microscope equipped with a water-immersion 40×/1.0 DIC objective and was continuously perfused at 1.5-2.0 ml/min with oxygenated regular ACSF (34-35°C). CA1 pyramidal cells were visualized a Sensicam QE CCD camera. Whole-cell patch-clamp recordings were obtained using a MultiClamp 700B amplifier and Clampex 10.2 software. The signal was low-pass filtered at 2 kHz and sampled at 5 kHz with a DigiData 1440A interface. Recording electrodes had tip resistances ranging from 4–6 MΩ when filled with internal solution, which contained (in mM): 110 K-gluconate, 25 KCl, 10 HEPES, 0.2 EGTA, 2 MgATP, 0.3 NaGTP, 10 Na2- creatinine phosphate, 0.1% biocytin, pH 7.2-7.3. E_{Cl^-} was -43 mV. Of note, E2 modulation of IPSCs was never observed when recording with a CsCl-based internal solution, possibly owing to interference with postsynaptic G protein-coupled signaling. Series resistance (12–25 MΩ) was compensated (70%) and monitored every minute with 10 ms, 3 mV steps from a holding potential of –70 mV. Cells were excluded from analysis if more than a 20% change in series resistance occurred during a recording. Data were not corrected for junction potential (14 mV). $GABA_A$ receptor-mediated IPSC**s** were evoked via a glass bipolar stimulating electrode (tip: 10–20 µm) placed in the CA1 pyramidal cell layer 30–150 µm from the recording electrode, and were recorded in the presence of 6,7-dinitroquinoxaline-2,3-dione (DNQX; 10 µM), DL-2-amino-5-phosphonopentanoic acid (DL-AP5; 50 µM), and CGP55845 (1 µM). Evoked IPSCs were blocked by the $GABA_A$ receptor antagonist SR 95531 (2 µM) applied at the end of each experiment.

For evoked IPSCs, paired stimulations (0.1 msec duration) were delivered at 0.067 Hz with a 100 msec inter-stimulus interval using a WPI stimulus isolation unit. We recorded IPSCs elicited by activation of multiple or single interneuron axons, as assessed by a stimulus-response curve for each cell. Putative unitary IPSCs were identified by: 1) 10% decrements or increments of stimulus intensity did not change

mean IPSC amplitude, 2) response latency and/or shape did not change over the course of the experiment, and 3) decreasing stimulus intensity by >15% led to an abrupt and total failure of responses. For compound IPSCs, stimulus intensity was adjusted to evoke an IPSC of approximately 50% of the maximum amplitude. For unitary IPSCs, stimulus intensity was set at 110–120% of the threshold intensity. Stimulus intensity was then kept constant throughout a recording. Drug and/or E2 effects were assessed by averaging IPSCs recorded during the last 2 min before drug application to those recorded during the last 2 min in each condition. Failures were included in analysis of pharmacological effects. E2-responsive experiments were identified using a threshold of 25% change in IPSC amplitude (see Results). For DSI, cells were depolarized to 0 mV for 2 sec.

All chemicals were purchased from Sigma, except for CGP55845, SR 95531, PPT, DPN, AM251, JNJ 16259685, MPEP, CPCCOEt (Tocris Bioscience), JZL 184 (Cayman Chemical Co.), and GDPβS (EMD Chemicals, Tocris). Stock solutions were prepared in DMSO or ddH_2O, and were diluted in aCSF on the day of recording to the final concentrations indicated. Control aCSF contained an equivalent concentration of DMSO (<0.1%).

Data are reported as mean ± SEM. Statistical comparisons were made using paired Student's *t*-tests and $p < 0.05$ was taken as indicating a statistically significant effect ($*p < 0.05$ and $**p < 0.01$).