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Estradiol regulates markers of synaptic plasticity in the hypothalamic ventromedial nucleus and amygdala of female rats

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ABSTRACT

Ovarian hormones act in multiple brain regions to modulate specific behaviors and emotional states. For example, ovarian hormones promote female sexual receptivity in the hypothalamic ventromedial nucleus (VMH) and modulate anxiety in the amygdala. Hormone-induced changes within the VMH include structural modifications, such as changes in dendritic spines, dendrite length and the number of synapses. In some situations, dendrite remodeling requires actin polymerization, which depends on phospho-deactivation of the enzyme cofilin, or the ionotropic AMPA-type glutamate receptors, especially the GluA1 and GluA2 subunits. The present experiments used immunohistochemistry to test the hypothesis that ovarian hormone-induced neural plasticity in the VMH and amygdala involves the regulation of phospho-cofilin, GluA1 and GluA2. These proteins were assessed acutely after estradiol administration (0.5, 1.0 and 4.0 h), as well as three days after hormone treatment. Both brain regions displayed rapid (4.0 h or less) and transient estradiol-induced increases in the level of phospho-cofilin. At the behaviorally relevant time point of three days, differential changes in AMPA receptor subunits were observed. Using Golgi impregnation, the effect of estradiol on amygdala dendrites was examined. Three days after estradiol treatment, an increase in the length of dendrites in the central nucleus of the amygdala was observed. Thus, estradiol initiates structural changes in dendrites in both the VMH and amygdala associated with an early phospho-deactivation of cofilin, followed by dynamic, brain region-specific changes in AMPA receptor composition.

Introduction

Levels of the ovarian hormones estradiol and progesterone fluctuate in females across the lifespan, rising during puberty and cycling during adulthood, with a temporary hiatus during lactation. Ovarian hormone levels provide an internal indicator of reproductive status, driving an array of complementary behavioral changes. Some brain targets of estradiol control reproductive functions. For example, the hypothalamic ventromedial nucleus (VMH), an area rich in estrogen receptor (ER)-α and estradiol-induced progestin receptors, controls female mating behavior in a broad array of species, ranging from lizards to mammals (as reviewed in Griffin and Flanagan-Cato, 2011). A remarkable aspect of female ovarian cycles is the concomitant remodeling of neuronal connectivity in the VMH and other brain regions (reviewed in Ferri and Flanagan-Cato, 2012). In particular, ovarian hormones modify the dendrite morphology of VMH neurons, including dendritic spine density and dendrite length (Calizo and Flanagan-Cato, 2000; Griffin and Flanagan-Cato, 2011); however, the cytoskeletal mechanisms that underlie these changes in neuronal structure remain unknown.

In addition to regulating reproductive behavior, the ovarian cycle recalibrates the motivational–emotional state of the female brain. For example, ovarian hormones modulate anxiety-related behaviors (Zimmerberg and Farley, 1993); specifically, estradiol exerts anxiolytic actions (Frye and Walf, 2004; Hiroi and Neumaier, 2006; Leret et al., 1994; Nomikos and Spyraki, 1988). Anxiety-like behaviors vary across the estrous cycle in female rats, with the lowest levels of anxiety-like behaviors occurring during proestrus (Frye et al., 2000; Mora et al., 1996). Likewise, systemic and intra-amygdalar injections of estradiol reduce anxiety-like behaviors in ovariectomized females (Frye and Walf, 2004; Mora et al., 1996). Two regions within the amygdala of particular interest are the basolateral area (BLA) and the central nucleus of the amygdala (CeA). The BLA receives glutamatergic inputs (LeDoux, 1998) and relays information to the adjacent CeA via glutamatergic synapses. The CeA controls species-specific threat responses via its outflow to autonomic, neuroendocrine and motor control sites. Estradiol treatment changes activity in the amygdala, which expresses ER-β (Corodimas and Morrell, 1990; DonCarlos et al., 1991; Osterlund et al., 2000; Shughrue and Merchenthaler, 2001). For example, estradiol treatment blunts the effect of chronic foot shock stress on the activation

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of specific signaling molecules in the CeA (Gerrits et al., 2006). Moreover, treatment with glucocorticoids directly into the CeA, which is known to increase anxiety-like behavior, is not anxiogenic in animals treated with an estrogen receptor (ER)-β agonist (Weiser et al., 2010). The underlying cellular mechanisms that mediate estradiol's anxiolytic effect in the amygdala are not known, and we propose that they may involve synaptic reorganization.

Dendrite remodeling requires structural modifications of the cytoskeletal protein β-actin, and spinous protrusions require high concentrations of β-actin (Kaech et al., 1997; Matus, 2000). The severing of filamentous actin is performed by the constitutively active enzyme cofilin, which is inactivated by phosphorylation. Thus, phospho-deactivation of cofilin is associated with elongation of actin filaments, which provides the protrusive motor for dendritic spine development (Bernstein and Bamburg, 2010). Although estradiol-induced changes in the dendrite morphology of neurons have been demonstrated in a variety of brain regions, namely the VMH, medial amygdala, hippocampus, and cortex (Cooke, 2010; Kramar et al., 2009; Srivastava et al., 2008; Woolley, 1998), little is known about the possible effects of ovarian hormones on the regulation of cytoskeletal proteins in the VMH, BLA and CeA.

The neurotransmitter most associated with dendrite remodeling is glutamate. In particular, one class of glutamate receptors, the AMPA ionotropic receptors, is critical for activity-based plasticity. AMPA receptors are multimeric channels, including the obligatory GluA1 and optional GluA2 subunits, which contribute to different functions (Lu et al., 2009). GluA1 is important for activity-based trafficking and basal synaptic transmission, whereas GluA2 allows for calcium conductance. Thus, these experiments sought to compare hormone-induced changes in actin regulation with changes in AMPA receptor subunit levels. We hypothesized that estradiol transiently modulates phospho-cofilin levels, with longer lasting effects on AMPA receptor subunits.

Materials and methods

Animals

Adult female Sprague–Dawley rats weighing 220–250 g were obtained from Taconic (Hudson, NY) and group-housed in plastic tubs (41 × 21 × 22 cm) with standard bedding. Rat chow and water were available ad libitum. The colony was maintained at 22 °C on a 12/12-hour reverse light/dark cycle, with lights off at 1000 h. Animals were allowed one week to acclimate before any procedures were performed. The Institutional Animal Care and Use Committee of the University of Pennsylvania approved all animal procedures.

Ovariectomy

Surgeries were performed under aseptic conditions. Animals were bilaterally ovariectomized under general anesthesia: 90 mg/kg ketamine and 9 mg/kg xylazine (both intraperitoneally, and both Phoenix Pharmaceutical, Inc., St. Joseph, MO). The lumbar incisions were closed with absorbable chromic gut sutures and suture clips (Fine Science Tools, Foster City, CA). After surgery, rats were given yohimbine (2.1 mg/kg, ip, Lloyd Laboratories, Shenandoah, IA) to counteract the anesthesia produced by xylazine. Animals were given one week of monitored recovery before experiments commenced.

Hormone treatment

One week after surgery, the animals were randomly assigned to one of three hormone treatment groups: vehicle (veh), estradiol benzoate (EB) alone, or EB combined with progesterone (EBP) on a four-day cycle shown to induce lordosis. The veh control group received injections of sesame oil (100 μl, sc) on Days 1 and 2, followed by propylene glycol (100 μl, sc) on Day 4. The two other treatment groups were primed with 17β-estradiol benzoate (EB; 10 μg in 100 μl sesame oil, sc, Sigma, St. Louis, MO) on Days 1 and 2. On Day 4, the EB group received vehicle (propylene glycol; 100 μl, sc) and the EBP group, progesterone (500 μg in 100 μl propylene glycol, sc, Sigma). Four hours after the injection on Day 4, the animals were perfused (see below).

Another set of OVX females was given a single injection of veh or EB and perfused 0.5 h, 1.0 h, or 4.0 h later.

Enzyme linked immunosorbent assay (ELISA)

Animals were injected sc with vehicle or EB (see above). Thirty or 60 min later, 2 mm was cut from the tip of the tail to obtain blood, which was collected in Microvette CB 300 Z capillary collection tubes (Sarstedt, Newton, NC). Blood was centrifuged at 4 °C for 15 min to separate plasma. Duplicates of 25 μl of plasma were run according to the Calbiotech estradiol ELISA kit (Spring Valley, CA).

Perfusion and sectioning

Animals were anesthetized (90 mg/kg ketamine and 9 mg/kg xylazine, intraperitoneal) and perfused transcardially through the ascending aorta with 100 ml saline followed by 200 ml 4% paraformaldehyde (Electron Microscopy Sciences, Fort Washington, PA). The brains were isolated, post-fixed in paraformaldehyde overnight at 4 °C, then submerged in 30% sucrose in 0.1 M phosphate buffer. Coronal sections encompassing the VMH were cut on a freezing microtome (Olympus, Tokyo, Japan) into four serial sets of 40-μm-thick slices. Sections were stored in cryoprotectant at −20 °C.

Immunohistochemistry

Sections were washed in Tris-buffered saline (TBS; pH 7.4), 0.3% hydrogen peroxide (H_2O_2, Fisher Scientific; Fair Lawn, NJ), and TBS again. They were then incubated according to the manufacturer's concentration suggestions with Anti-Glutamate Receptor 2 antibody (1:200, AB1768, rabbit, Millipore, Temecula, CA), Anti-Glutamate Receptor 1 antibody (1:150, AB1504, rabbit, Millipore, Temecula, CA), or anti-phospho-cofilin antibody (1:100, Cell Signaling, Danvers, MA) in TBS with 0.2% TritonX-100 and 3% normal goat serum (Jackson ImmunoResearch; West Grove, PA) for 2 days at 4 °C. After several TBS washes, sections were incubated in Biotin-SP-AffiniPure Goat Anti-Rabbit IgG (Jackson ImmunoResearch) in TBS with 0.2% TritonX-100 and 3% normal goat serum (Jackson ImmunoResearch) for 2.5 h at room temperature. Following several TBS washes, sections were incubated in avidin–biotin–peroxidase complex (Elite Standard ABC kit, Vector Laboratories, Burlingame, CA) for 1.5 h. Sections were then treated with 50 mM Tris containing 3,3-diaminobenzidine (DAB, 0.2 mg/ml, Fisher Scientific; Fair Lawn, NJ), nickel sulfate (25 mg/ml, Sigma-Aldrich, St. Louis, MO), and 0.025% H_2O_2 for 25 min. Finally, sections were washed in TBS, mounted on gel-coated slides, air-dried, and coverslipped with Permount mounting media (Fisher Scientific).

The GluA1 and GluA2 antibodies were previously validated for their specificity at these concentrations in immunohistochemistry (Das et al., 2008).

Immunohistochemical analysis

Images were acquired using a digital camera (Diagnostic Instruments, Sterling Heights, MI, model RTKE) with an Olympus Optical BX50 microscope (Olympus, Tokyo, Japan). All images were captured using the same exposure time in order to preserve relative staining intensity. An experimenter naïve to the treatment groups then analyzed the images. A standardized shape encompassing the vlVMH, dmVMH, LFC, BLA, or CeA, as identified by a rat brain atlas (Paxinos and