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Low luteinizing hormone enhances spatial memory and has protective effects on memory loss in rats

Shira G. Ziegler [a], Janice E. Thornton [a,b,*]

[a] Neuroscience Department, Oberlin College, 119 Woodland Street, Oberlin, OH 44074, USA
[b] Biology Department, Oberlin College, 119 Woodland Street, Oberlin, OH 44074, USA

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ABSTRACT

Though several studies have suggested that estradiol improves hippocampal-dependent spatial memory, the effects of other hormones in the hypothalamic–pituitary–gonadal axis on memory have largely been ignored. Estradiol and luteinizing hormone (LH) are generally inversely related and LH may significantly affect spatial memory. Ovariectomized (ovx) rats treated with Antide (a gonadotropin releasing hormone receptor antagonist) had low LH levels and showed enhanced spatial memory, comparable to treatment with estradiol. Antide-treated ovx females retained spatial memory longer than estradiol-treated ovx females. Deficits in spatial memory are a primary symptom of neurodegenerative disorders including Alzheimer's disease (AD). Treatment with Antide prevented spatial memory deficits in a neurotoxin-induced model typical of early AD. These data suggest that memory impairments seen in female rats after ovariectomy or women after menopause may be due to high LH levels and that a reduction in LH enhances memory. These results also implicate an LH lowering agent as a potential preventative therapy for AD.

Introduction

Several studies have suggested that estradiol modulates cognitive function (Cholerton et al., 2002) and that it improves hippocampal-dependent spatial memory in female rats and mice (Dohanich, 2002; McLaughlin et al., 2008; Frick, 2009). However, there is generally an inverse relationship between estradiol and luteinizing hormone (LH). Ovariectomy (ovx) leads to high levels of LH which are reversed with estradiol treatment. High LH levels can impede memory. Berry et al. (2008) showed that administration of an LH homologue interferes with the enhanced spatial memory seen in ovx estradiol-treated rats. In addition, transgenic mice that overexpress LH show disruption in cognitive performance even though they have high estradiol levels (Casadesus et al., 2007). These data indicate that estradiol's improvement of cognitive function and memory may at least partly be due to the hormone's suppression of LH.

It has also been suggested that estradiol ameliorates the development of the neurodegenerative disorder Alzheimer's disease (AD). Deficits in spatial memory are a primary symptom of AD (delpolyi et al., 2007). The hippocampus encodes and initially stores experience-dependent spatial information (Pastalkova et al., 2006; Martin and Clark, 2007) and is one of the main brain structures damaged by AD (West et al., 1994, 2000). There is a striking sex bias for AD development; with a 2.5-fold higher prevalence of AD in women than men even when age is controlled for (Jorm et al., 1987; McGonigal et al., 1993; Carlson et al., 2001). This is consistent with an earlier loss of reproductive function and sex steroids in women than men, and has led investigators to focus on the role of estradiol and other estrogens in the pathogenesis of AD.

Despite evidence supporting a protective role of estrogens in memory decline and AD (Xu et al., 1998; Manly et al., 2000; Hruska and Dohanich, 2007), studies based on the Women's Health Initiative have failed to find protective effects of estrogen on age-related memory decline in postmenopausal women (Rapp et al., 2003). It has been suggested that the lack of an effect of estrogen in these studies may be because the hormone treatment was delayed (Maki, 2006; Sherwin, 2006) as there is evidence in both rats and women that long-term estrogen deprivation diminishes the beneficial effects of estrogen on cognitive functioning (Frick, 2009). Long-term estrogen deprivation also diminishes the ability of replacement estrogen to decrease the elevated LH that occurs in response to ovx or menopause (King et al., 1987; Rossmanith et al., 1994). Because of these and other inconsistencies, other hormones in the hypothalamic–pituitary–gonadal (HPG) axis are being explored as possible factors in the memory decline and pathogenesis associated with AD.

LH may be involved in the development of AD. Short et al. (2001) found that circulating gonadotropin levels were 2-fold higher in AD patients compared with controls. Individuals with Down's syndrome have elevated LH levels and a higher chance of contracting AD-like lesions and symptoms early in life (Oliver and Holland, 1986; Mann, 1988). Elevated β-amyloid levels have been implicated in AD pathogenesis and LH has been found to increase β-amyloid in

* Corresponding author. Neuroscience Department, Oberlin College, 119 Woodland Street, Oberlin, OH 44074, USA.
E-mail address: jan.thornton@oberlin.edu (J.E. Thornton).

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neuroblastoma cells (Bowen et al., 2004). Berry et al. (2008) also showed that rats treated with memory-impairing levels of an LH homologue had elevated brain β-amyloid species. The prolonged suppression of LH in both wildtype mice (Bowen et al., 2004) and an AD mouse model (Casadesus et al., 2006) has been shown to decrease β-amyloid.

The present studies explored whether the memory enhancement associated with estradiol might be at least partly due to its negative feedback effects on LH. We hypothesized that decreasing LH would enhance spatial memory in ovx rats, even in the absence of estradiol. We also explored whether lowering LH would ameliorate the memory loss seen in a rat model of AD. We used the AD rat model developed by Dornan et al. (1993) and utilized by Hruska and Dohanich (2007). The model is based on the infusion of the neurotoxins β-amyloid and ibotenic acid into the hippocampus and mimics the pathology of early AD. If decreased LH prevented memory loss in a neurotoxin-infused, AD model, then this would implicate an LH lowering agent as a possible treatment for AD.

Materials and methods

Animals

Female Sprague–Dawley rats were derived from the breeding of animals purchased from Hilltop Animal Laboratories. All rats were weaned at 4 weeks and housed in same-sex groups. Prior to experimentation, rats were moved to groups of three in plastic cages measuring 27.9 cm × 20.3 cm × 17.8 cm. Rats were kept in a 72 °F temperature-controlled room with a 14 h light, 10 h dark cycle (lights on at 6:00am) with ad libitum access to Purina Labdiet and water. All procedures met NIH standards and were approved by the Oberlin College Institutional Animal Care and Use Committee.

Hormones and drugs

All rats were ovx under continuous isoflorane anesthesia (0.75 l/minO_2; 3% anesthetic). A Silastic capsule that contained either estradiol or cholesterol was implanted subcutaneously between the shoulder blades at time of ovx. Capsules were constructed from Silastic tubing (1.57 mm i.d., 3.18 mm o.d.; Dow Corning; sealed with wood dowel and silicone). Estradiol capsules were filled with a crystalline mixture of 1 part estradiol-17β (Sigma, St. Louis, MO) to 3 parts cholesterol (Sigma, St. Louis, MO) and control capsules were filled with cholesterol. The length of estradiol or cholesterol within the tubing measured 5 mm. These 25% estradiol capsules maintain circulating estradiol at low physiological levels (25 pg/ml) typical of gonadally intact females (Luine and Rodriguez, 1994; Daniel et al., 1997). Silastic capsules were equilibrated in 0.9% saline for 24 h prior to implantation. All behavioral testing occurred within 2 to 3 months of implantation. Functionality of estradiol implants was verified through vaginal smears 3 months after initial implantation: predominately cornified cells were found in rats with estradiol capsules and primarily leukocytes in rats with control capsules (Jones et al., 1961).

The gonadotropin releasing hormone receptor antagonist, Antide (Bachem, Torrance, CA), was dissolved in distilled water. Antide (1 mg/kg) or water vehicle was injected subcutaneously, both at a volume of 1 ml/kg. Antide has been shown to significantly decrease serum LH and FSH levels (Weinbauer and Nieschlag, 1993). The dose of Antide was chosen based on experiments that found that a single 1 mg/kg Antide injection significantly decreased testosterone levels in adult male rats within 24 h (Habenicht et al., 1990) and a single 1.25 mg/kg Antide injection significantly decreased serum LH levels to almost undetectable levels in a non-human primate model within 12 h and lasted for at least 96 h (Weinbauer and Nieschlag, 1993).

Neurotoxin infusions

Intrahippocampal infusions of neurotoxins or vehicle were administered as previously described to mirror the effects of human AD (Dornan et al., 1993; Hruska and Dohanich, 2007). Briefly, females were anesthetized with sodium pentobarbital (40 mg/kg) or continuous isoflorane exposure (0.75 l/min O_2; 3% anesthetic) and bilaterally injected in the dorsal hippocampus with a 1 μl solution containing aggregated β-amyloid (4 μg/μl; Sigma, St. Louis, MO) and ibotenic acid (1 μg/μl; Sigma, St. Louis, MO), or 0.9% saline vehicle. β-Amyloid (1–42) was dissolved in sterile distilled water and pre-aggregated by *in vitro* incubation for 4 days at 37 °C. Ibotenic acid was dissolved in PBS and added to the β-amyloid aggregate immediately before surgery. Solutions were administered by stereotaxic injection with a 26-gauge needle Hamilton microsyringe over 1 min into the dorsal hippocampus (3.3 mm posterior, 1.5 mm lateral to bregma, and 4.0 mm ventral to the skull surface, Paxinos and Watson, 2007). The needle remained in position for an additional 2 min after infusion and then the procedure was repeated on the contralateral hippocampus and the incision closed.

Open field habituation and locomotor activity tests

Before rats were tested in an object location memory task, females were habituated over a 3-day period to an 80 cm × 80 cm × 30 cm open field arena with the floor marked in a grid comprised of 10 cm × 10 cm squares as previously described by Berry et al. (2008). External visual cues, including a 55.8 cm × 69.8 cm white cross on a black background on one wall, remained stationary throughout habituation and behavioral testing. On the first day of habituation, groups of three rats were placed in the arena with wood shavings for 20 min. On day two of habituation, groups of three rats were placed in the arena for 20 min without wood shavings. On the third day of habituation, rats were placed individually in the arena without wood shavings for 5 min each.

To determine locomotor activity levels, rats were placed individually in the arena without wood shavings for 5 min and the number of 10 cm spaced lines crossed was recorded. There is an inverse relationship between activity and anxiety levels, and differences in locomotor activity in an open field have been used to determine differences in anxiety (Bronstein, 1972).

Object location memory test

This task was adapted from an object location memory test developed by Ennaceur et al. (1997) and modified by Mumby et al. (2002). This memory task relies on the natural exploratory behavior of the rat; a rat that notices an object has been moved will investigate that object longer than an object that remains in the same place (Ennaceur and Meliani, 1992). Testing took place in an open field arena described above, covered in wood shavings. Each test consisted of two trials: an exposure trial and a test trial. During the exposure trial, two identical objects were placed in two quadrants of the open field 20 cm from each wall. The rat was introduced into the arena and allowed to explore for 5 min. After the exposure trial, the rat was returned to its home cage. Both objects were wiped with 70% ethanol and wood shavings were mixed to disrupt olfactory cues.

After a designated intertrial delay (refer to specific experimental procedures), the rat was returned to the open field for a test trial. For this trial, one object was moved to a new quadrant in one of two counterbalanced configurations (see Berry et al., 2008). The rat was introduced and allowed to explore for 3 min and the amount of time each object was explored was recorded. The amount of time the rat explored each object was measured in seconds according to the criteria established by Ennaceur and Delacour (1988). Briefly, the rat's nose had to be no more than 2 cm away from the object for it to be considered an exploration. If the rat was standing with its front paws