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Human chorionic gonadotropin (a luteinizing hormone homologue) decreases spatial memory and increases brain amyloid-β levels in female rats

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Abstract

Numerous studies have suggested that estradiol (E) improves spatial memory as female rats with E perform better than those without E. However there is an inverse relationship between E and luteinizing hormone (LH) levels and LH could play a role. We examined whether treatment with the LH homologue human chorionic gonadotropin (hCG), would impair spatial memory of adult E-treated female rats. In the object location memory task, ovariectomized (ovxed) rats treated with E and either a single high dose (400 IU/kg) or a lower repeated dose of hCG (75 IU/kg hourly for 8 h) showed spatial memory disruption compared to ovxed rats treated with estradiol alone. Impairment was attributed to memory disruption as performance improved with shortened delay between task exposure and testing. Tests on another spatial memory task, the Barnes maze, confirmed that hCG (400 IU/kg) can impair memory: although E+veh treated animals made significantly fewer hole errors across time, E+hCG-treated did not. In humans, high LH levels have been correlated with Alzheimer's disease (AD). Because brain amyloid-beta (Aβ) species have been implicated as a toxic factor thought to cause memory loss in AD, we analyzed whether hCG-treated animals had increased Aβ levels. Levels of Aβ from whole brains or hippocampi were assessed by Western blot. hCG treatment to E-implanted females significantly increased soluble Aβ40 and Aβ42 levels. These results indicate that high levels of LH/hCG can impair spatial memory, and an increase in brain Aβ species may account for the memory impairment in hCG-treated rats.

Keywords: Memory; Spatial memory; Luteinizing hormone; Amyloid-β; Estradiol; Hippocampus; Alzheimer's

Introduction

Numerous studies have suggested that estradiol (E) is needed for optimal levels of spatial memory by females, particularly on tasks that depend on working memory (for review see Dohanich 2002). For example, ovariectomy (ovx) decreases spatial memory, and ovxed female rats treated with E perform better than ovxed females treated with vehicle on a number of spatial memory tasks that utilize working memory including the Morris water maze, object place memory task, and the radial arm maze (Bimonte and Denenberg, 1999; Gibbs, 1999; Luine et al., 1998; Luine et al, 2003; Packard, 1998; Sandstrom and Williams, 2001; Simpkins et al., 1997).

Although E has been considered responsible for these spatial memory effects, there is an inverse relationship between estradiol and the level of luteinizing hormone (LH) and it could be LH rather than E that is responsible. LH is secreted from the anterior pituitary and E normally exerts negative feedback inhibition of LH release. Removal of E by ovx causes a subsequent increase in serum LH and replacement with estradiol lowers LH (Freeman et al., 1976; Wise and Ratner, 1980). The role of LH in ovxed animals has not been addressed in studies that have examined E's effect on spatial memory. If LH does play a role then high LH in the presence of high E should block E's effects on spatial memory and low levels of LH in the presence of low E should still facilitate spatial memory.

There is evidence that LH can act at the hippocampus to affect behavior. LH and its homologue human chorionic gonadotropin (hCG) can cross the blood-brain barrier (Knowles, 1972; Lukacs et al., 1995; Oliver et al., 1977) and the highest density of LH/

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hCG receptors in the central nervous system is found in the hippocampus (Lei et al., 1993), a brain area that is essential for memory. LH can modulate neuronal activity in the hippocampus (Gallo et al., 1972) and can modify hippocampus-associated behaviors in rats such as taste neophobia, locomotion and rearing (Lukacs et al., 1995).

Suggestive of a role for LH in memory, elevated LH has been implicated as a risk factor in Alzheimer's disease (AD). AD is the most common form of clinical dementia and is characterized by selective neurodegeneration in the hippocampus and progressive memory loss leading to cognitive decline. Reports find serum LH to be significantly higher in individuals with AD compared to age matched controls (Bowen et al., 2000; Short et al., 2001; Hogervorst et al., 2004; though for contradictory evidence see Hogervorst et al., 2003). Patients with Down syndrome have elevated levels of LH throughout life and develop cognitive impairment and AD-like lesions early in life (Mann, 1988; Oliver and Holland, 1986). Moreover, immunohistochemical analysis suggests that the amount of LH is increased in the cytoplasm of pyramidal neurons in AD brains (Bowen et al., 2002).

Brain amyloid proteins have been implicated in the pathophysiology of AD and it is possible that LH may increase amyloid-β (Aβ) levels. In the hippocampus amyloid protein precursor, a single pass transmembrane protein, is cleaved by secretases into Aβ40 and Aβ42. Increases in the secretion and aggregation of Aβ molecules are thought to be responsible for cell toxicity and memory impairment in AD (Koh et al., 1990; Yankner et al., 1990; Mattson et al., 1992, 1993; Morgan et al., 2000; Naslund et al., 2000). A series of studies have shown that levels of soluble Aβ correlate with the degree of cognitive impairment and disease progression in animal models and AD subjects (Kuo et al., 1996; McLean et al., 1999; Mucke et al., 2000; Naslund et al., 2000) and increasing evidence has suggested that soluble non-fibrillar Aβ rather than the insoluble fibrillar counterpart is important for the pathophysiology of the disease (Walsh, 1999; Lambert et al., 2000). In addition, LH has been found to cause elevated levels of Aβ in neuroblastoma cells *in vitro* (Bowen et al., 2004), and the prolonged suppression of LH in both normal mice (Bowen et al., 2004) and an AD mouse model (Casadesus et al., 2006) has been shown to decrease Aβ load and aggregates, respectively, *in vivo*.

In the present studies we explored whether elevated levels of LH may contribute to the memory changes previously attributed to E. hCG was used in place of LH due to it's availability. Both hCG and LH have a similar structure and act at the same receptor to produce similar effects (Lei and Rao, 2001; Loosfelt et al., 1989; McFarland et al., 1989). More specifically, we examined whether elevated hCG would disrupt E's enhancement of spatial memory in female rats. We then tested whether elevated hCG, at levels that impair spatial memory, would increase Aβ40 or Aβ42 in the brain.

Materials and methods

Animals

Adult female Sprague–Dawley rats derived from the breeding of animals purchased from Hillltop Animal Laboratories were used. All were weaned at 4 weeks and housed in same-sex groups. Just prior to surgery animals were housed in groups of 3 in plastic cages measuring 27.9 cm × 20.3 cm × 17.8 cm. Animals were kept on a 14 hour light: 10 hour dark cycle (7:00 pm lights off) with ad libitum access to Purina Labdiet and water. All behavioral testing took place between 7:30–9:30 pm under red light. All procedures met NIH standards and were approved by the Oberlin College Institutional Animal Care and Use Committee.

Hormones

All rats were ovariectomized (ovxed) under isoflorane anesthesia and implanted subcutaneously with a silastic capsule that had been equilibrated in saline overnight prior to implantation. Silastic capsules (15 mm long with an inner diameter of 1.57 mm, and an outer diameter of 3.18 mm), were plugged with wood and sealed on either end with elastomer (Dow Corning) so that each capsule contained either 5 mm of estradiol-17β (E: Sigma) or no hormone (blank). Because the permeability, diameter, wall thickness, and concentration gradient are constant in the capsule, the length of the capsule determines the diffusion rate (Legan et al., 1975). Estradiol capsules of this size provide constant circulating levels of approximately 75 pg/ml estradiol for over 1 year (Legan et al., 1975; Karsch et al., 1973). Purified hCG (Prospec-Tany) was reconstituted in deionized water (400 IU/ml) and stored in aliquots at −20 °C for 1–4 weeks. hCG or water vehicle was injected intraperitoneally.

Open field habituation and anxiety/activity tests

Prior to the object location memory task, females were habituated to the open field arena. The arena was 80 cm × 80 cm × 30 cm with the floor marked in a grid comprised of 10 cm × 10 cm squares. A large black and white extra-maze cue (a white 35 × 35 cm cross on a black 70 × 55 cm background) was located on one wall outside of the open field box. On the first day of habituation, groups of 2–4 rats were placed in the arena (with wood shavings) for 20 min. On day 2 of habituation groups of 2–4 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 (with wood shavings) for 5 min.

To determine anxiety and activity levels, rats were placed individually in the arena (without wood shavings) for 5 min and the number of 10 cm squares crossed (a measure of activity) and the number of seconds spent in the center of the open field arena (a measure of anxiety: rats that are anxious will spend less time in the center of an open field) were recorded. Rats were tested the day after habituation (before hCG or vehicle) and immediately after the first Object Location Memory Test, approximately 7–9 h after hCG or vehicle administration.

Object Location Memory Test

This task is based on one described by Ennaceur et al. (1997). Testing took place in the 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 equidistant from the two objects with its head facing the wall and allowed to explore for 5 min. The amount of time the rat explored each object (i.e. the rat's nose was at most 2 cm away from the object) was measured in seconds (Ennaceur and Delacour, 1988). Afterwards the rat was returned to its home cage. Shavings in the open field were mixed to eliminate odor trails and objects were cleaned with 70% ethanol. After a designated intertrial delay (see specific experiments), 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, the rat was introduced into the same side of the arena as for the exposure trial and allowed to explore for 3 min and the amount of time each object was explored was recorded. Animals underwent 2–3 behavioral tests, separated by intervals of 4 days. Data from all tests were averaged for each rat. Testers were blind to the treatment group they scored.

Barnes maze

The Barnes maze tested the ability of rats to use fixed spatial cues to locate an escape box that allowed the rats to escape a lighted platform (see Barnes,