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Galanthamine plus estradiol treatment enhances cognitive performance in aged ovariectomized rats

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ABSTRACT

We hypothesize that beneficial effects of estradiol on cognitive performance diminish with age and time following menopause due to a progressive decline in basal forebrain cholinergic function. This study tested whether galanthamine, a cholinesterase inhibitor used to treat memory impairment associated with Alzheimer's disease, could enhance or restore estradiol effects on cognitive performance in aged rats that had been ovariectomized in middle-age. Rats were ovariectomized at 16–17 months of age. At 21–22 months of age rats began receiving daily injections of galanthamine (5 mg/day) or vehicle. After one week, half of each group also received 17ß-estradiol administered subcutaneously. Rats were then trained on a delayed matching to position (DMP) T-maze task, followed by an operant stimulus discrimination/reversal learning task. Treatment with galanthamine + estradiol significantly enhanced the rate of DMP acquisition and improved short-term delay-dependent spatial memory performance. Treatment with galanthamine or estradiol alone was without significant effect. Effects were task-specific in that galanthamine + estradiol treatment did not significantly improve performance on the stimulus discrimination/reversal learning task. In fact, estradiol was associated with a significant increase in incorrect responses on this task after reversal of the stimulus contingency. In addition, treatments did not significantly affect hippocampal choline acetyltransferase activity or acetylcholine release. This may be an effect of age, or possibly is related to compensatory changes associated with long-term cholinesterase inhibitor treatment. The data suggest that treating with a cholinesterase inhibitor can enhance the effects of estradiol on acquisition of a DMP task by old rats following a long period of hormone deprivation. This could be of particular benefit to older women who have not used hormone therapy for many years and are beginning to show signs of mild cognitive impairment. Potential mechanisms for these effects are discussed.

Introduction

Animal studies show that ovariectomy and estrogen replacement significantly affect the structure and function of hippocampal and cortical circuits with corresponding effects on the performance of a wide variety of cognitive tasks (Daniel and Bohacek, 2010; Fernandez et al., 2008; Sandstrom and Williams, 2001, 2004; Spencer et al., 2008; Wallace et al., 2006; Woolley, 2007). Studies in humans likewise have demonstrated beneficial effects of estrogen therapy on specific cognitive tasks in younger surgically menopausal and perimenopausal women, particularly in the realm of verbal memory and executive functioning (Sherwin and Henry, 2008). A recent study also shows that women who experience an early menopause are at significantly greater risk for age-related cognitive decline and dementia, and this risk is mitigated by early estrogen therapy (Shuster et al., 2010). Animal studies, however, suggest that many of the beneficial effects of estradiol on cognitive performance diminish with age and time following ovariectomy when initiation of therapy is delayed (Daniel et al., 2006; Gibbs et al., 2009; Markowska and Savonenko, 2002; Talboom et al., 2008). This is consistent with human trials reporting limited benefit when estrogen therapy is initiated in older women. In fact, several large trials including the very large Women's Health Initiative Memory Study (WHIMS), have reported either no beneficial effect or increased harm for women receiving hormone therapy (HT) in old age (Resnick et al., 2006; Shumaker et al., 2003, 2004). These findings have given rise to the critical period hypothesis, which proposes that estrogen therapy must be initiated within a window of time following menopause in order to produce beneficial effects on brain function and cognition (Daniel and Bohacek, 2010; Maki, 2006; Sherwin, 2009).

We hypothesize that this window of opportunity is defined, at least in part, by the function of cholinergic projections originating in the septum and nucleus basalis magnocellularis, and innervating the hippocampus and cortex (Gibbs, 2010). Acetylcholine release in the hippocampus and cortex increases wakefulness and attention, plays a role in stimulus-reward behavior, and has important effects on

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learning (Parikh and Sarter, 2008; Pepeu and Giovannini, 2010; Schliebs and Arendt, 2006). The function of cholinergic afferents declines with age (Baskerville et al., 2006; Fischer et al., 1992b) as well as with specific neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease (Lanari et al., 2006; Linstow and Platt, 1999; Smith et al., 1999). This is demonstrated by decreases in the number and size of cholinergic neurons in the MS, DBB, and NBM (Altavista et al., 1990; Fischer et al., 1989, 1992a; Mesulam et al., 1987; Stroessner-Johnson et al., 1992), decreases in high affinity choline uptake (Kristofiková et al., 1992; Sherman and Friedman, 1990), acetylcholine release (Araujo et al., 1990; Moore et al., 1996; Takei et al., 1989; Wu et al., 1988), and cholinergic synaptic transmission (Taylor and Griffith, 1993). These neurons also are adversely affected by loss of ovarian function as demonstrated by decreases in ChAT and TrkA expression beyond the effects of normal aging (reviewed in Gibbs, 2010). Studies also show that some of the increases in hippocampal plasticity and cognitive performance that are produced by estradiol in young rats are lost or attenuated by cholinergic deafferentation (Gibbs, 2007; Lam and Leranth, 2003; Rudick et al., 2003), or by pharmacological inhibition of cholinergic receptors (Daniel et al., 2005).

Recently we showed that in rats that had been ovariectomized as young adults, treating with donepezil (a cholinesterase inhibitor) at advanced age restored beneficial effects of estradiol on acquisition of a delayed matching-to-position (DMP) T-maze task (Gibbs et al., 2009). Likewise, the combination of donepezil + estradiol significantly enhanced acquisition as well as delay-dependent memory in young rats with partial loss of septal cholinergic neurons (Gibbs et al., 2011). These findings suggest that addition of a cholinesterase inhibitor can enhance or restore beneficial effects of estradiol that have been compromised by a decrease in basal forebrain cholinergic function. The purpose of the present study was to extend these findings by testing whether galanthamine, another cholinesterase inhibitor commonly used to treat Alzheimer's disease, can restore effects of estradiol on cognitive performance in aged ovariectomized rats and to determine whether the behavioral effects are associated with effects on cholinergic function.

Materials and methods

Animals

Forty-eight female Fisher/Brown Norway rats were purchased at 15 months of age from Harlan Sprague–Dawley, Inc. out of the National Institute on Aging rodent colony. Rats were maintained on a 12 hour:12 hour light/dark schedule with lights on at 0700 and with free access to food and water. All procedures were consistent with PHS guidelines on the care and use of laboratory animals, and with the approval of the University's Institutional Animal Care and Use Committee.

Treatments

At 16–17 months of age, rats were anesthetized with a combination of ketamine and xylazine and the ovaries were surgically removed. Rats received ketofen (1 mg, 2×/day s.c.) for three days following surgery to alleviate discomfort. At 21–22 months of age (5 months after ovariectomy), rats began receiving daily injections of galanthamine hydrobromide (Gal, 5 mg/kg; Tocris, Inc.) or sterile saline delivered i.p. Injections continued until rats were euthanized. Galanthamine (4aS,6R,8aS)-4a,5,9,10,11,12-Hexahydro-3-methoxy-11-methyl-6H-benzofuro[3a,3,2-ef] [2]benzazepin-6-ol) is a competitive, reversible cholinesterase inhibitor that is approved for the treatment of mild to moderate Alzheimer's disease and vascular dementia. Galanthamine inhibits acetylcholinesterase in the brain with an IC50 of approximately 2.8–3.2 μM for the frontal cortex and the hippocampus (Thomson et al., 1991) and has a half-life of approximately 7 h. In addition to its anticholinesterase activity, galanthamine acts as an allosteric enhancer at multiple nicotinic receptor subtypes (Samochocki et al., 2003; Schilstrom et al., 2006). The dose of 5 mg/kg was selected based on studies by Geerts et al. (2005) suggesting that doses in the range of 1.5–5.0 mg/kg in rats produce optimal brain concentrations for the allosteric enhancing effect, and on a study by Woodruff-Pak et al. (2003) showing that a dose of 3 mg/kg reversed the effects of the nicotinic receptor blocker mecamylamine on conditioned eye blink response in rabbits. Injections were administered at the end of the day (~5:00 PM) to avoid the effects of mild stress on learning (Fitz et al., 2006). One week after the beginning of treatment, half of each group (Gal or sterile saline) received a silastic capsule (6 mm length, 1.98 mm I.D., 3.18 mm O.D) packed with 3 mm of 100% powdered 17ß-estradiol (Sigma-Aldrich, Inc.), implanted s.c. The remaining rats received an empty capsule as a control.

DMP training

One week after receiving the silastic capsule, rats were food restricted to 85% body weight and then trained on a delayed matching to position (DMP) T-maze task as described previously (Gibbs et al., 2009, 2011; Gibbs and Johnson, 2007). Rats were trained to traverse the maze and enter the goal arms by using a series of 6 forced "choice" trials per day for at least 4 days, each of which was rewarded with 4 food pellets (45 mg, formula 5TUM, Test Diets, Inc.). Right and left arms were alternated randomly in order to avoid the introduction of a side bias. DMP training was then performed in trial pairs. Each rat received 8 trial pairs per day. The first trial of each pair consisted of a "forced" choice in which one goal arm was blocked, requiring the rat to enter the open arm to receive a food reward (2 pellets). The rat was then immediately returned to the start box. All arms of the maze were wiped with 50% ethanol, and both goal arms were opened for the second trial. A choice was defined as an animal placing both front legs and at least one rear leg into a goal arm. Returning to the same arm as the previous "forced" trial resulted in food reward (4 pellets). Entering the opposite arm resulted in no reward and confinement to the arm for 10 s. During each day of training, four arms on the right and four arms on the left were selected in random order for the "forced" trials. After each trial pair, the rat was returned to its cage for 5–10 min. Each rat continued to receive 8 trial pairs per day until it reached a criterion of 15/16 correct choices over two consecutive days, or until it received 30 days of training.

Post-criterion testing

All rats that reached criterion also received post-criterion testing. One day after reaching criterion, animals received a probe trial during which the T-maze was rotated 180° (relative to extramaze cues) between the forced and open trials. This probe trial was done to determine whether rats were utilizing a place strategy (i.e., were utilizing extramaze cues) or a response strategy (i.e., were utilizing internal or kinetic cues) to perform the task. An animal using extramaze cues is expected to turn in the opposite direction (i.e., enter the opposite physical arm now located in the same position of the room as the goal arm during the "forced" trial), while an animal using internal or kinetic cues would be expected to turn in the same direction (i.e., enter the same physical arm as during the "forced" trial) even though the arm occupies a different position in the room. Selecting the arm located in the same position of the room as during the "forced" trial was assigned a score of 0, while selecting the opposite arm was assigned a score of 1. One day after the probe trial, animals received four days of additional DMP training to assess delay-dependent effects on performance. On these days animals received 8 trial pairs/day and the delay between the "forced" and "open" trial was increased