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Long-term treatment with estrogen and progesterone enhances acquisition of a spatial memory task by ovariectomized aged rats☆

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Abstract

Female Sprague–Dawley rats were ovariectomized at 13 months of age. Four groups received different regimens of estrogen or estrogen plus progesterone replacement beginning either immediately, 3 months, or 10 months after ovariectomy and were compared with non-hormone-treated controls. Eight to twelve months after ovariectomy, animals were trained on a delayed matching-to-position (DMP) spatial memory task. Long-term treatment with estrogen or estrogen plus progesterone significantly enhanced acquisition of the DMP task by aged animals after long-term loss of ovarian function. Weekly administration of estrogen and progesterone was at least as effective as, if not more effective than, continuous treatment with estrogen alone. In addition, treatment initiated 3 months, but not 10 months, after ovariectomy was as effective at enhancing DMP acquisition as continuous estrogen treatment initiated immediately after ovariectomy, suggesting a window of opportunity after the loss of ovarian function during which hormone replacement can effectively prevent the effects of aging and hormone deprivation on cognitive function. These findings suggest that repeated treatment with estrogen and progesterone initiated within a specific period of time after the loss of ovarian function may be effective at preventing specific negative effects of hormone deprivation on brain aging and cognitive decline.

Keywords: Hormone replacement; Aging; Alzheimer's disease; Learning

1. Introduction

Recent studies suggest that estrogen replacement therapy may help to reduce the risk [1,20,22,42,55] and severity [21,40,41] of Alzheimer's-related dementia in postmenopausal women. In addition, studies have shown that estrogen replacement can enhance measures of verbal memory and associative learning in young and older women [44–48]. These findings raise important questions about the potential of hormone replacement therapy to enhance cognitive performance and to reduce or prevent age-related cognitive decline in postmenopausal women.

The neurobiological mechanisms that underlie these effects are currently unknown, but most likely reflect effects of estrogen on the survival, connectivity, and function of specific neural systems. We and others have demonstrated that, in rats, cholinergic neurons in the medial septum (MS), the vertical limb of the diagonal band of Broca (DBB), and the nucleus basalis magnocellularis (NBM), are significantly affected by physiological fluctuations in circulating gonadal steroids, and that acute estrogen or estrogen plus progesterone replacement can significantly enhance the functional status of these neurons as evidenced by increases in choline acetyltransferase (ChAT) [10,13,19,31], high affinity choline uptake [39,52], and acetylcholine release [18]. These neurons are the primary source of cholinergic innervation to the hippocampus and cerebral cortex and have been shown in many studies to play an important role in learning, memory, and attention (see [11,56,60] for reviews). These neurons are also negatively affected by Alzheimer's disease (AD) and their loss is thought to contribute to Alzheimer's-related dementia [29] as well as to less severe age-related cognitive decline.

Recently we demonstrated that in 19-month-old rats, long-term (6 months), but not short term (3 months) loss of ovarian function produced significant decreases in the levels of ChAT and nerve growth factor receptor (trkA) mRNA in the MS and NBM relative to age-matched, gonadally intact controls [14]. This suggests that long-term loss of ovarian

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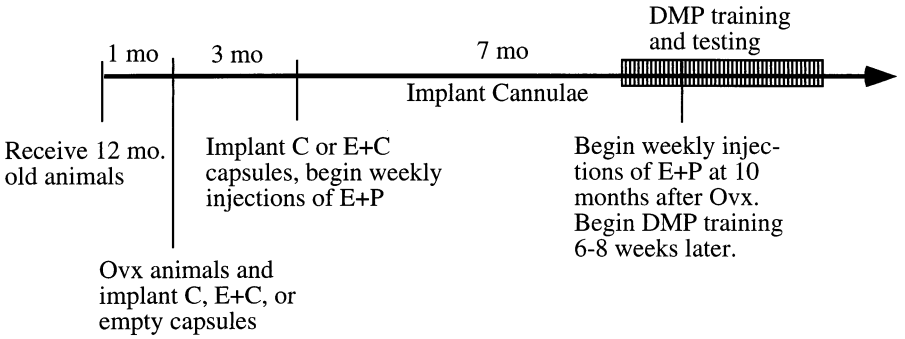

Fig. 1. Diagram summarizing the experimental design and the timing of major interventions.

function can have negative effects on the cholinergic neurons that go beyond the effects of normal aging. These effects may, in turn, contribute to a decline in cholinergic function and to an increased risk for age- and AD-related dementia. We have hypothesized that one mechanism by which hormone replacement may help to enhance cognitive processes and reduce Alzheimer's-related cognitive decline is by enhancing and sustaining the functionality of cholinergic projections to the hippocampus and cortex and thereby preserving function in the presence of hippocampal and cortical impairment.

By using a delayed matching-to-position (DMP) spatial memory task, we recently demonstrated that, in young ovariectomized animals, chronic estrogen replacement resulted in an increased rate of learning and decreased impairment after intrahippocampal injections of the muscarinic receptor antagonist scopolamine [12]. These findings are consistent with the idea that estrogen replacement can increase learning and reduce deficits associated with hippocampal cholinergic impairment. Whether long-term treatment with estrogen or estrogen plus progesterone has similar effects in older animals, and whether the effects vary as a function of the hormone replacement regimen or the time elapsed between the loss of ovarian function and the initiation of hormone treatment is currently unknown. In the present study, we used the DMP task to examine the ability of different regimens of hormone replacement to enhance the performance of older animals when administered at different times after the loss of ovarian function.

2. Methods

One hundred eleven 12 month old female Sprague–Dawley rats (retired breeders) were purchased from Harlan Sprague–Dawley Laboratories and housed in pairs on a 12 h light : 12 h dark cycle with food and water available ad lib. All animals were ovariectomized in house at 13 months of age and divided into five treatment groups. One group received continuous low dose estrogen plus cholesterol beginning immediately after ovariectomy (OE, $n = 24$). The estrogen was delivered via a 5 mm silastic capsule (0.058" ID, 0.077" OD, Dow Corning Corp., Midland, MI, USA) containing 25% 17-β-estradiol (E) crystals and 75% cholesterol (Sigma, Inc., St. Louis, MO, USA) implanted subcutaneously (s.c.). These capsules produce mean circulating levels of E of approximately 15 to 25 pg/ml serum. A second group received continuous low dose estrogen plus cholesterol beginning 3 months after ovariectomy (O3E, $n = 24$). Every 2 months, the estrogen capsules were moved slightly by palpating the capsules through the skin. This was done to help prevent the development of fibroids around the capsules that could interfere with estrogen delivery. A third group received weekly injections of estrogen and progesterone (10 μg of 17-β-E2 in 0.1 cc sesame oil injected s.c. followed 48 h later with 500 μg progesterone in 0.1 cc sesame oil s.c.) beginning 3 months after ovariectomy (O3EP, $n = 24$). These injections produce mean E levels of approximately 50 pg/ml serum and mean P levels of approximately 10 ng/ml serum when measured 5 h after administration [10,13,15]. A fourth group received weekly injections of estrogen and progesterone (10 μg of 17-β-E2 in 0.1 cc sesame oil injected s.c. followed 48 h later with 500 μg progesterone in 0.1 cc sesame oil s.c.) beginning 10 months after ovariectomy (O10EP, $n = 13$). Controls received either capsules containing cholesterol or sham surgery (OC, $n = 24$). All capsules and sham surgeries were performed using metophane anesthesia (Schering Plough Animal Health Corp. Omaha, NE, USA). DMP training and testing began 8 to 12 months after ovariectomy and after at least 6 to 8 weeks of treatment in the O10EP-treated animals (see Fig. 1).

2.1. Cannula implantation

At least 1 month before training, all animals received 22 Ga. stainless steel guide cannulae (cannulae length = 4.5 mm, intercannulae separation = 3.8 mm, Plastics One, Inc., Roanoke, VA, USA) implanted bilaterally into the hippocampus (coordinates: −3.3 mm from Bregma, 1.9 mm lateral, −3.3 mm from dura). Animals were anesthetized with a mixture of ketamine (50 mg/ml) and xylazine (10 mg/ml) [0.25 cc/250 g.b.w. intraperitoneal (i.p.); A.J. Buck, Inc., Baltimore, MD, USA] and placed into a standard