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Multiple clinically relevant hormone therapy regimens fail to improve cognitive function in aged ovariectomized rhesus monkeys

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ABSTRACT

Preclinical studies in aged, surgically-menopausal rhesus monkeys have revealed powerful benefits of intermittent estrogen injections on prefrontal cortex–dependent working memory, together with corresponding effects on dendritic spine morphology in the prefrontal cortex. This contrasts with the inconsistent effects of hormone therapy (HT) reported in clinical studies in women. Factors contributing to this discrepancy could include differences in the formulation and sequence of HT regimens, resulting in different neurobiological outcomes. The current study evaluated, in aging surgically menopausal rhesus monkeys, the cognitive effects of 4 HT regimens modeled directly on human clinical practice, including continuous estrogen treatment opposed by progesterone. None of the regimens tested produced any cognitive effect, despite yielding physiologically relevant serum hormone levels, as intended. These findings have implications for the design of regimens that might optimize the benefits of hormone treatment for healthy aging, and suggest that common HT protocols used by women may fail to result in substantial cognitive benefit, at least via direct effects on the prefrontal cortex.

1. Introduction

The effects of ovarian hormones on cognition are age dependent. That is, loss of circulating ovarian hormones early in the lifespan has relatively little effect on cognitive function, but produces more dramatic and widespread impairment later in life, around the time of natural menopause (e.g., Hao, et al., 2007; Markowska and Savonenko; 2002, Rapp, et al., 2003; Voytko, 2000; Voytko, 2002). These studies, as well as at least some observational investigations in women, support the notion that hormone therapy (HT) after menopause benefits cognitive function. However, the Women's Health Initiative Memory Study (WHIMS) found that treatment of older women (aged 65 to 79 years at study onset) with conjugated equine estrogen (CEE) alone, or CEE plus progestin (medroxyprogesterone acetate [MPA]) had no beneficial effect on global cognitive function, was associated with decline in global cognitive function in some women, and increased the risk of mild cognitive impairment (MCI) and Alzheimer's disease (Espeland, et al., 2004; S.R. Rapp, et al., 2003; Shumaker, et al., 2004; Shumaker, et al., 2003). Considerable discussion around these findings has centered on the limitations of the WHIMS design (Craig, et al., 2005; Maki, 2004; Sherwin and Henry, 2008), including a "healthy user" bias in women who elect HT, confounds associated with other age-related health problems, such as obesity and hypertension, the specific hormone formulations tested (Zhao and Brinton, 2006; Nilsen and Brinton 2003), and the fact that women in the WHIMS began HT after a prolonged period of ovarian hormone deprivation (the window of opportunity hypothesis).

Studies in animal models mitigate some of the challenges associated with studying the relationship between ovarian hormones and cognition in aging humans. Nonhuman primates, specifically rhesus monkeys, are well suited for modeling the relationship between neuroendocrine and cognitive aging in humans. Many aspects of reproductive physiology are similar between rhesus monkeys and women, notably including the periodicity of the normal menstrual cycle and the late-life onset of menopause (Gilardi, et al., 1997; Walker, 1995). Impairment in cognitive function accompanies menopause in rhesus monkeys (Roberts, et al., 1997), which, as in humans, includes prefrontal cortex dysfunction (Weber, et al., 2012).

Our goal in the present study was to investigate whether HT strategies that incorporate continuous E dosing, as well as progesterone, benefit cognition in aged, ovariectomized (OVX) rhesus monkeys. An initial study in aged OVX rhesus monkeys (mean age,

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22 years) found strong cognitive benefits of cyclic estradiol therapy, consisting of an injection of 100 μg of estradiol cypionate (E) every 21 days (Rapp, et al., 2003). Although this regimen is FDA-approved for use in women, continuous E supplementation is more common because it avoids symptoms associated with fluctuating E levels, such as hot flashes. Thus, one goal was to determine whether continuous E administration provided behavioral benefits similar to those previous seen for cyclic E therapy. A second issue relates to the modulatory effect of progesterone (P). Most women who receive HT after menopause take a combination of E and P to counter the proliferative effects of E on the uterine endometrium. Whereas data from rodents on the memory effects of P are conflicting (reviewed in Frick, 2009), a recent study in aged rhesus monkeys (mean age, 19.7 years) found that cyclic treatment with P did not modify the cognitive effects of a continuous E treatment regimen supplemented by E injections (Voytko, et al., 2008; Voytko, et al., 2009). Thus, we also determined the effects of combining continuous or cyclic P with E treatment. These studies used the same model, modified to better mimic natural ovarian cyclicity, and testing procedures that were sensitive to a beneficial effect of cyclic E treatment on cognition (Rapp, et al., 2003).

If continuous E or E treatments combined with P fail to modulate cognition in older OVX monkeys, this would strongly suggest that the effectiveness of HT is determined by the specific hormones given (E alone vs. E and P together) as well as the timing of dosing (cyclic vs. continuous). In this way, we aimed to determine how the specific characteristics of clinically relevant hormone replacement regimens contribute to key cognitive outcomes, independent of other factors that might also influence the effects of HT.

2. Methods

2.1. Overview of experimental timeline

Aged, female, behaviorally naive rhesus monkeys (late teens through mid-20s) were screened for inclusion in the study according to several criteria: the absence of previous experimental surgical manipulations making them ineligible for inclusion in a subsequent research protocol involving survival surgery, per United States Department of Agriculture regulations; no previous memory testing, long-term neuropharmacological or dietary intervention, pre- or perimenopausal reproductive status, based on ovarian hormone profiles (determined by urinary hormone metabolite assays); and satisfactory physical health with an absence of opthalmic impairments (including cataracts or macular degeneration), severe arthritis, or gross physical abnormalities. Thus, monkeys that began the study were in excellent physical health and had intact ovarian function at the beginning of the experimental protocol.

Monkeys selected for inclusion in the study were trained to criterion on the delayed response (DR) task at 0 and 1-second delays, ovariectomized, and recovered from ovariectomy for between 6 and 12 weeks. During this time, serum hormone assays were conducted to verify effectiveness of ovariectomy. Each monkey was then assigned to 1 of 5 treatment groups balanced for their preoperative performance on DR: vehicle (VEH, n = 7), continuous estrogen by Silastic implant (E_{CONT}, n = 9), continuous estrogen and daily oral progesterone (E_{CONT}+P_{CONT}, n = 9), continuous estrogen and intermittent (cyclic) progesterone (E_{CONT}+P_{CYC}, n = 8), or intermittent injections of estrogen and intermittent progesterone (E_{CYC}+P_{CYC}, n = 9). Beginning 1 month after initiation of hormone treatment, behavioral testing resumed, consisting of reacquisition of DR at a 1-second delay, then proceeding through DR testing with increasing delays, DR with distraction, delayed non–matching-to-sample (DNMS) training to criterion at a 10-second delay, DNMS with increasing delays, DNMS with distraction, object discrimination (OD) training, and OD testing with distraction. Two of these tasks (DR and DNMS) have documented sensitivity to ovarian hormone treatment (injection of estrogen every 21 days) in aged, ovariectomized monkeys (Rapp, et al., 2003). Versions of all 3 tasks with distraction were also included to test the hypothesis that beneficial effects of hormone treatment might be mediated by a general effect of ovarian hormones on a cognitive process engaged by multiple memory tests, in this case reduced distractibility. The median duration of treatment (time from initiation of hormone treatment post-OVX to perfusion) was 533 days (range, 209–757 days), including monkeys that did not complete all phases of behavioral testing (see below).

2.2. Subjects

The experiments were performed at the California National Primate Research Center (CNPRC) under protocols approved by the University of California, Davis Institutional Animal Care and Use Committee. Forty-two aged (17.7–25.7 years old at ovariectomy; mean age ± SD = 20.9 ± 1.9 years), female, behaviorally naive rhesus monkeys (*Macaca mulatta*) were studied. Thirty-four monkeys completed all phases of behavioral testing. Completion of the entire postoperative testing battery took about 16 months on average (mean, 15.8 months; range, 12.6–21.8). The other 8 monkeys did not finish the entire protocol. Three completed DR testing through the distraction phrase of the task (see below) and were euthanized during DNMS testing, 1 for suspected endometriosis, 1 because of a rapidly-growing tumor on the mandible, and 1 because of poor recovery after treatment of an infection at the implant site. One monkey completed DR testing as well as DNMS (delays only), and then died of a ruptured kidney. One completed delay and distraction testing phases of both DR and DNMS, and was then euthanized because of weight loss. Two completed all phases of testing except object discrimination with distraction, and were euthanized for poor physical condition or for diarrhea and poor appetite. The eighth monkey completed all behavioral testing in good health but exhibited motivational problems during object discrimination testing; because she would not reliably complete daily test sessions on this task, her data for this task were excluded. Because behavioral testing was suspended when health problems became apparent (in the 7 monkeys that were euthanized) and responding in the test apparatus was prompt until that time (and until OD testing for the eighth monkey), their behavioral data were included in analyses for tasks for which complete data were available. The pattern of results does not change if all data are excluded from monkeys that failed to complete the entire behavioral protocol because of health reasons. The monkeys that were euthanized were in the E_{CONT} group (n = 4), E_{CONT}+P_{CONT} group (n = 2), or E_{CYC}+P_{CYC} group (n = 1). Thus, at the end of testing, the number of subjects in each group were as follows: vehicle (n = 7), E_{CONT} (n = 5), E_{CONT}+P_{CONT} (n = 7), E_{CONT}+P_{CYC} (n = 8), and E_{CYC}+P_{CYC} (n = 8). Most monkeys (36/42) were pair housed during the day and separated at night. Night time separation facilitated monitoring of food intake and allowed collection of individual animal urine samples for hormone assays.

The average lifespan of captive rhesus macaques is less than 25 years (Peters, et al., 1996; Tigges, et al., 1988), and previous studies demonstrate that prominent behavioral, neurobiological, and endocrine signs of aging are not observed in monkeys younger than 19 or 20 years of age (Bachevalier, et al., 1991). The range of ages for the monkeys was therefore selected as the most appropriate for addressing the specific questions under investigation, given that our aim was to define the effects of HT manipulations when