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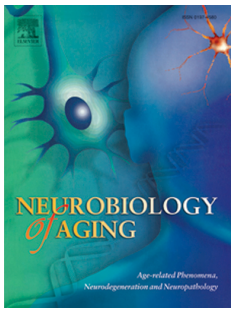

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Effect of short-term DHEA supplementation on serum and hippocampal estrogen concentrations in perimenopausal female rhesus macaques

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ABSTRACT

The hippocampus of rhesus macaques expresses genes that encode key enzymes involved in the intracrine conversion of dehydroepiandrosterone (DHEA) to estradiol. Therefore, it is plausible that supplementary DHEA may enhance hippocampal estradiol concentrations and help to compensate for the marked postmenopausal attenuation of circulating estrogen levels. To test this hypothesis, we used LC-MS/MS to measure estradiol and estrone concentrations in the serum and hippocampus of young and old perimenopausal female rhesus macaques, as well as old perimenopausal females that received daily DHEA (5 mg) oral supplementation for 1 week. Despite lower concentrations of these estrogens in the serum of the older animals, their concentrations in the hippocampus did not show any obvious differences due to age or to DHEA supplementation. The results suggest that *de novo* estrogen synthesis in the brain may compensate for the perimenopausal loss of estrogens in the circulation even without supplemental DHEA.

1. Introduction

Like women, female rhesus macaques show a marked age-associated decline in circulating estradiol concentrations (Downs & Urbanski, 2006), and several monkey studies have demonstrated the therapeutic potential of estrogen supplementation on age-associated cognitive decline (e.g., Kohama et al., 2016; Lacreuse, 2006; Rapp et al., 2003; Voytko et al., 2008). Although it is unclear if other steroids can exert similar pro-cognitive effects a possible candidate is DHEA, a highly abundant steroid produced mainly by the adrenal cortex. In the circulation of humans and rhesus macaques, DHEA exists mainly in its sulfated form (DHEAS), which shows a marked age-associated attenuation (e.g., Downs et al., 2008; Labrie et al., 1998; Sorwell & Urbanski, 2013; Urbanski & Sorwell, 2012). Importantly, the rhesus macaque hippocampus expresses genes that encode key enzymes in the intracrine conversion of DHEA to estradiol (Sorwell *et al.* 2012). In theory, therefore, there is potential for cognitive centers of the brain to maintain elevated estradiol concentrations even after menopause, especially if supplementary DHEA is provided to serve as a precursor. To test this hypothesis, we used LC-MS/MS to examine the concentrations of two endogenous estrogens, estrone (E_1) and estradiol (E_2), in the serum and hippocampus of young, old, and DHEA-supplemented old female rhesus macaques.

2. Materials and methods

2.1. Experimental animals

This study was approved by the Oregon National Primate Research Center (ONPRC) Institutional Animal Care and Use Committee and used 20 female rhesus macaques (*Macaca mulatta*). The animals were housed indoors under controlled environmental conditions: 24° C temperature; 12-h light and 12-h dark photoperiods (lights on at 07:00 h), and were cared for by the ONPRC Division of Comparative Medicine in accordance with the National Research Council's *Guide for the Care and Use of Laboratory Animals.* Daily meals at ~0800 h and ~1500 h (LabDiet High Protein Monkey Chow, St. Louis, MO, USA) were supplemented with fresh fruits or vegetables; fresh drinking water was available *ad libitum.*

2.2. Tissue collection and hormone measurement

Eight old (average age = 23.9 ± 0.45 years) females received oral DHEA supplementation (5 mg; inside a marshmallow treat at 07:45 h each morning) for 1 week, including the morning of necropsy. Preliminary tests found this administration paradigm to mimic the youthful circulatory patterns of DHEAS, characterized by daily morning peaks generally in the range of 160 – 400 ng/ml 1-2 hours after DHEA administration (Sorwell et al., 2017). A terminal blood sample was collected at necropsy (at ~ 10: 00 h) from the DHEA-treated animals, as well as from five young adult females (average age = 12.6 ± 0.73 years) and seven DHEA-naïve old females (average age = 23.6 ± 0.62 years). All of the animals were sacrificed on days when their endogenous serum E_2 levels were expected to be basal; i.e., in the cycling animals this was during the early follicular phase. Euthanasia was performed by trained ONPRC Division of Comparative Medicine veterinarians following guidelines established by the Panel on Euthanasia of the American Veterinary Medical Association. Serum from the terminal blood samples was stored at -20° C until time of assay. The brains were flushed with 0.9% saline and the hippocampus flash frozen in liquid N_2 and stored at -80° C.

E_1 and E_2 concentrations were assayed in the serum and hippocampal samples using LC-MS/MS, as previously described (Supplementary Material). Serum cortisol, DHEA, DHEAS, 5α-dihydrotestosterone, E_2 and testosterone were also assayed, either by ECL, ELISA or RIA (Fig. S1).

3. Results

One-way ANOVA, followed by Tukey's HSD, revealed significant group differences in serum E_2 levels with higher levels in the young animals as compared to the old controls and DHEA-supplemented animals ($p < 0.01$) (Fig. 1A). Similarly, there was a trend (defined as $0.05 < p < 0.10$) for serum E_1 concentrations (Fig. 1A) with higher levels in the young as compared to old control ($p = 0.095$, Cohen's d = 0.77) and DHEA-supplemented animals ($p = 0.076$, Cohen's d = 0.81). In contrast, no obvious group differences were observed in hippocampal concentrations of either E_1 or E_2 (Fig. 1B), although there was a trend for higher E_1 concentrations in the young compared to DHEA-treated old animals ($p = 0.095$, Cohen's d = 0.20). For reference, the effect of DHEA on serum levels of various steroid hormones derived from DHEA are shown in Fig. S1. Serum DHEAS levels were significantly higher in the DHEA supplemented animals than in the age matched controls ($p < 0.01$), suggesting rapid sulfation of the exogenous DHEA, and possibly some conversion to testosterone and 5αDHT but not E_2. Serum cortisol levels were similar in all of the animal groups.

4. Discussion

As expected, LC-MS/MS showed that young animals had significantly higher concentrations of E_2 in their serum compared to the old animals, a finding that was corroborated by the ECL assay results; a similar trend was also observed for E_1. However, age-related differences in hippocampal E_1 and E_2 concentrations were less obvious, suggesting that the old females were still able to synthesize sufficient estrogen *de novo* in their cognitive brain centers. Whether or not this mechanism involves intracrine conversion of DHEA to E_1 and E_2 is unclear because there was no difference in hippocampal estrogen concentrations between the DHEA-treated old animals and the age-matched controls. Surprisingly, the hippocampal concentrations of E_1 appeared to be much higher than those of E_2. The physiological relevance of this finding is unclear, as E_1 appears to lack some of the beneficial pro-cognitive effects of E_2 and may even impair learning and memory (Barha & Galea, 2010; Engler-Chiurazzi et al., 2012; McClure et al., 2013).

There are several possible explanations for why DHEA supplementation failed to increase hippocampal E_1 and E_2 concentrations. First, the duration of the DHEA treatment may have been too short, or the single daily dose

of DHEA that we used (5 mg) may have been too low to overcome its rapid conversion to DHEAS, with limited amounts of DHEA left available to serve as a substrate for sex-steroid synthesis in the brain. Second, because the level of endogenous DHEA was still relatively high in the circulation of the old animals, it may have provided sufficient substrate to maintain maximum intracrine conversion to estrogen within the brain, masking any additional contribution from exogenous DHEA. Third, the mechanism responsible for the intracrine conversion of DHEA to E_1 and E_2 in the hippocampus may have been less effective in the old animals, a view that is supported by previous studies showing that expression of *HSD3B1/2*, a gene that encodes a key enzyme in the DHEA- E_2 conversion, itself shows an age-related decrease in the rhesus macaque hippocampus (Sorwell et al., 2012). Taken together, the results help to explain why DHEA supplementation appears to be largely ineffective at improving cognitive function in older monkeys and humans, with most clinical studies failing to demonstrate any significant benefit (e.g., Davis et al., 2011; Panjari & Davis, 2010; Scheffers et al., 2015; Sorwell et al., 2017).

Disclosure statement

The authors confirm that no actual, or potential, conflicts of interest exist.

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Fig. 1. Measurement of estrogen concentrations in the serum (**A**) and hippocampus (HPC) (**B**) of female rhesus macaques. Estrone (E_1) and estradiol (E_2) were extracted from serum and HPC of young, old, and old DHEA-treated female rhesus macaques. Values represent means ± SEMs. ** $p < 0.01$, compared to the *Young* group.