ELSEVIER

Neurobiology of Aging 33 (2012) 1487.e1–1487.e13

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

# Perimenopausal regulation of steroidogenesis in the nonhuman primate

Krystina G. Sorwell[a,b], Steven G. Kohama[a], Henryk F. Urbanski[a,b,c,*]

[a] Division of Neuroscience, Oregon National Primate Research Center, Beaverton, OR, USA
[b] Department of Behavioral Neuroscience, Oregon Health and Science University, Portland, OR, USA
[c] Department of Physiology and Pharmacology, Oregon Health and Science University, Portland, OR, USA

Received 4 January 2011; received in revised form 3 May 2011; accepted 8 May 2011

**Abstract**

Human aging is characterized by a marked decrease in circulating levels of dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEAS), hormonal changes associated with cognitive decline. Despite beneficial effects of DHEA supplementation in rodents, studies in elderly humans have generally failed to show cognitive improvement after treatment. In the present study we evaluate the effects of age and estradiol supplementation on expression of genes involved in the de novo synthesis of DHEA and its conversion to estradiol in the rhesus macaque hippocampus. Using reverse transcription polymerase chain reaction (RT-PCR) we demonstrate the expression of genes associated with this synthesis in several areas of the rhesus brain. Furthermore, real-time PCR reveals an age-related attenuation of hippocampal expression level of the genes *CYP17A1, STS*, and *3BHSD1/2*. Additionally, short-term administration of estradiol is associated with decreased expression of *CYP17A1, STS, SULT2B1*, and *AROMATASE*, consistent with a downregulation not only of estrogen synthesis from circulating DHEA, but also of de novo DHEA synthesis within the hippocampus. These findings suggest a decline in neurosteroidogenesis may account for the inefficacy of DHEA supplementation in elderly humans, and that central steroidogenesis may be a function of circulating hormones and menopausal status.


*Keywords:* Aging; Dehydroepiandrosterone; Hormone replacement; Menopause; Neurosteroidogenesis

## 1. Introduction

Human aging is associated with several physiological and cognitive changes, but the underlying etiology is poorly understood. Many of these age-associated disorders and pathologies are even more pronounced in females, due to the marked decrease of circulating estradiol ($E_2$) concentrations that occurs around the time of menopause. Consequently, common therapies developed for postmenopausal women involve estrogen replacement. In the brain, ovarian steroids are known to increase synaptic plasticity (Brann et al., 2007) and may improve cognition in postmenopausal women (Fillit et al., 1986; Tang et al., 1996). However, due to potential health risks associated with estrogen-based hormone replacement therapy (HRT) (Manson et al., 2003), there is need for safer alternative therapies to help alleviate postmenopausal disorders, such as cognitive decline.

One potential alternative therapy involves the adrenal steroid dehydroepiandrosterone (DHEA) and its ester, DHEA-sulfate (DHEAS), both of which decline with age in humans (Labrie et al., 1997). Importantly, administration of these steroids (DHEA and/or DHEAS [referred to collectively as DHEA/S]) to old mice has shown promise in restoring cognitive function to a level observed in young animals (Flood and Roberts, 1988; Markowski et al., 2001). However, as mouse and rat adrenal glands do not produce measurable levels of circulating DHEA/S, they may not be ideal models for human aging. In humans, high baseline levels of DHEA/S are associated with increased longevity in men, and in elderly women they have been associated with better cognitive performance (Davis et al., 2008; Sanders et al., 2010). As DHEA/S treatment carries fewer risks than estrogen re-

* Corresponding author at: Division of Neuroscience, Oregon National Primate Research Center, 505 NW 185th Avenue, Beaverton, OR 97006, USA. Tel.: +1 503 690 5306; fax: +1 503 690 5384.
*E-mail address:* Urbanski@ohsu.edu (H.F. Urbanski).

0197-4580/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
10.1016/j.neurobiolaging.2011.05.004

placement (Labrie et al., 2003; Panjari et al., 2009), supplementation with this hormone may represent a relatively safe alternative therapy compared with traditional forms of HRT. Despite promising associations with cognition, however, clinical studies in the elderly have failed to detect significant cognitive benefits of DHEA/S supplementation (Grimley-Evans et al., 2006). The reason for this is unclear.

One potential mechanism responsible for the procognitive effects observed in rodents involves local conversion of DHEA/S to $E_2$ (Sorwell and Urbanski, 2010). This phenomenon is a process known as intracrine conversion, or the conversion of a circulating prohormone to an active hormone that acts locally in an auto- or paracrine manner (Labrie, 1991). This steroid synthesis pathway involves the actions of the following enzymes, all of which are expressed in the rodent brain (Mellon and Griffin, 2002): sulfyl transferase (SULT2B1) and steroid sulfatase (STS) convert DHEA to DHEAS and vice versa, while 17$\beta$-hydroxysteroid dehydrogenase type 5 (17BHSD5), 3$\beta$-hydroxysteroid dehydrogenase types 1 and 2 (3BHSD1/2), and aromatase, are the primary enzymes involved in the central conversion of DHEA to $E_2$. This locally produced $E_2$ can have significant effects on hippocampal spine density and synapse frequency in vivo, suggesting metabolism of DHEA to $E_2$ is a likely mechanism of the observed cognitive effects of DHEA (Hajszan et al., 2004; Hirshman et al., 2004; Rune and Frotscher, 2005). Although well established in rodents, no studies have directly examined this steroidogenic mechanism in humans or nonhuman primates (NHPs). Thus, it is possible that an inability to convert DHEA/S to $E_2$ in the aged human brain underlies the lack of cognitive efficacy observed in clinical studies of DHEA/S supplementation (Sorwell and Urbanski, 2010). Additionally, an age-related decline in the de novo central synthesis of DHEA/S from cholesterol, without the contribution of peripheral hormone precursors, and a resulting loss of central $E_2$ may add to the cognitive effects of the loss of peripheral $E_2$ and serve as a potential target for therapeutic intervention.

The aim of the present study was to shed new light on neurosteroidogenesis in the aging primate brain, and to lay the foundation for potential novel therapies for age-associated cognitive decline. To overcome limitations associated with the rodent model of human aging, we utilized macaque monkeys. Specifically, our goal was to address the following questions: (1) Does de novo DHEA production in the brain, like that in the adrenal cortex, change with age; (2) could the lack of cognitive improvement in human DHEA replacement studies stem in part from reduced conversion of DHEA to $E_2$ in the hippocampus; and (3) does traditional HRT in adult females negatively impact neurosteroidogenesis in the hippocampus.

## 2. Methods

### 2.1. Experimental animals

This study was performed using plasma and tissues samples obtained from Japanese macaques (*Macaca fuscata*) and rhesus macaques (*M. mulatta*), maintained at the Oregon National Primate Research Center (ONPRC). The animals were fed a specially formulated monkey chow (Agway, Ithaca, NY, USA) twice daily, supplemented with fresh fruits and vegetables. Animal care was provided by the ONPRC Division of Animal Resources (DAR) in accordance with the NRC *Guide for the Care and Use of Laboratory Animals*, and the experiments were approved by the Oregon Health and Science University (OHSU) Institutional Animal Care and Use Committee.

Throughout the study, the age groups were defined as young adult (5–7 years), middle-aged (8–17 years), old (18–24 years), and oldest old (25 years and older).

### 2.2. Annual DHEAS and cortisol measurements

For several decades the ONPRC has maintained an outdoor colony of Japanese macaques, and during their annual physical examination plasma samples are collected from each animal (between 09:00 and 15:00 hours) and stored frozen at −80 °C. Using these archived samples and previously described assay procedures (Downs et al., 2008; Lemos et al., 2009), we examined the longitudinal plasma DHEAS and cortisol changes that occur across the adult lifespan of females from 6 to 29 years. Cortisol was measured using electrochemiluminescence (ECL) with the Elecsys 2010 Platform (Roche Diagnostics, Indianapolis, IN, USA). DHEAS was measured with radioimmunoassay (RIA) using a highly specific antibody against DHEA-17-(*O*-carboxymethyl) oxime-BSA (Endocrine Sciences, Tarzana, CA, USA) and [$^3$H] DHEAS (SA, 22 Ci/mM). Intra- and interassay coeffecients of variation were less than 10% for each assay and the assay detection limits were 3 ng/mL. A total of 172 measurements from 14 animals were performed for each hormone.

### 2.3. Twenty-four–hour plasma DHEAS and cortisol measurements

This experiment involved a total of 16 adult female rhesus macaques that were maintained indoors under a controlled lighting regimen comprising 12 continuous hours of light and 12 continuous hours of darkness per day (lights on a 07:00 hours). Daily menses records and plasma $E_2$ and progesterone ($P_4$) measurements were used to characterize the reproductive neuroendocrine status of these animals, as previously described (Downs and Urbanski, 2006). Accordingly, the animals were divided into the following 4 groups: young adult ($n = 5$), middle-aged ($n = 4$), old premenopausal ($n = 4$), and old perimenopausal ($n = 3$) animals. In the latter group, the animals either showed elongated (> 30