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Original Article

Effect of 17β estradiol on hippocampus region of aging female rat brain: Ultrastructural Study

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Abstract. Estradiol has direct membrane-mediated effects on neurons and its effects are both neuroprotective and neurotrophic. This hormone modulates brain development and aging and affects neurochemical systems which are affected in age-related cognitive decline, AD and other neuropsychiatric disorders. The aim of the present study was to determine the effect of 17β estradiol (E2) in hippocampus region of different age groups of rats. The changes in the hippocampus region of female rat brain of different age groups with and without E2 treatment were observed by transmission electron microscopy. Age dependent changes in myelin sheath, axon and cytoplasm membrane were observed with aging in control group rat brain but the E2 treated rats showed significantly stable myelin sheath, myelin axon and cytoplasm structure. Our results showed that E2 treatment significantly effects hippocampus brain region of aging rats. These analyses revealed that fundamental age-related changes in brain and estrogen have important implications when estrogen levels and hippocampus dependent functions decline.

Keywords: Brain aging, estradiol, estrogen receptor, hippocampus

Introduction

Aging is associated with a decline in metabolic function, affects the endocrine system by altering endocrine cells, the hormones produced by these cells, hormone receptors or post receptor processes in the target cells [1, 2, 3]. The process of aging presents itself with various alterations in physiological events. Advanced age is commonly associated with many of the serious neurological disorders and among them Alzheimer's Diseases (AD) is the most important age related neurological disorder in elderly population and there lies a difficulty in diagnosing and treating AD. Age-related impairment of functionality of the central nervous system (CNS) is associated with increased susceptibility to develop many neurodegenerative diseases. Increased oxidative stress in the CNS of aged animals is manifested by increased protein oxidation, which is believed to contribute to the age-related learning and memory deficits.

The ovarian steroid hormone 17β estradiol (E2) is an essential hormone which protects neurons against Aβ toxicity, oxidative stress and excitotoxicity [4-7]. Hormone replacement therapy (HRT) has effects on the brain at the functional, metabolic and neurotransmitter levels. Estrogens significantly affect the microstructure of brain regions, which are crucial to higher cognitive function and implicated in AD. Intracellular estrogen receptors (ER) are widespread and are found in the hippocampus, cerebral cortex, midbrain, brain stem, hypothalamus and pituitary gland. The distribution of ER is well established, being present in a high concentration in the hippocampus, hypothalamus, pituitary and amygdala. The action of estrogen has been shown to bind to cell membrane receptors, and affect the same second messenger systems used by growth factors and neurotransmitters [8]. However, estrogens also affect synaptic communication in brain regions involved in cognitive processing, such as the hippocampus [9], and these effects may be of particular importance in the context of aging, when both circulating estrogen levels change and hippocampus dependent functions decline [10]. The population of healthy neurons might be reduced, particularly in the hippocampus, leaving the brain with impaired ability to tolerate the neurodegenerative processes of aging and Alzheimer's disease. Males have higher brain levels of E2 than females, and this has been demonstrated for the hypothalamus, the major brain region undergoing sexual differentiation [11]. E2 is also synthesized de novo in the developing hippocampus and cortex. The amount of E2 measured in neonatal hippocampus and cortex is the same in males and females, and this may be the result of de novo local E2 synthesis [11]. The expression of ER-α and ER-β in the brain, in particular in the hippocampal formation, provides

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a potential target and mechanism for effects of estrogen in brain regions and circuits mediating cognitive processes such as memory [12-20]. The potential for E2 as a therapeutic neuroprotective agent in the aging brain has been of intense interest for over a decade. The aim of the present study was to investigate the age related changes in the hippocampal region of female rat brain of different age groups in the presence of E2 by means of the transmission electron microscopy (TEM).

Materials and Methods

Animals

The present study was conducted on female albino rats of the Wistar strain in different age groups (3, 12 and 24 months). Animals were maintained in the animal house facility of Jawaharlal Nehru University (JNU), New Delhi, India at a constant temperature of 25°C, humidity 55% and 12 h dark and light cycle. The animals were fed standard chow rat feed (Hindustan Leaver Ltd., India) and given tap water until the time of sacrifice. The Institutional Animal Ethics Committee (IAEC) of JNU approved all the animal experiments; all institutional guidelines for care of animals were followed.

Hormone Administration

Subcutaneous injections of E2 (0.1 μg/g body weight) were given daily for one month, to the aged rats (12 and 24 months old; n = 8 for each group). E2 was dissolved in propylene glycol in appropriate concentrations [21]. Control animals received an equal volume of vehicle. There was no treatment on the day of the sacrifice. Animals of all the groups were sacrificed and brains were isolated for further study.

Rat Perfusion

For rat perfusion 4% Paraformaldehyde- 2.5 % glutaraldehyde [22] has been used. We dissolved 4g Paraformaldehyde in 100ml of double distilled water at 60ºC with contunious stirring in a covered beaker to avoid evaporation. 1N NaOH was added drop wise with stirring until the solution become clear. To 100ml solution, 2.5% glutaraldehyde was added. Thereafter, the volume was made to 100ml with buffer solution. The pH of solution was adjusted to 7.4. Paraformaldehyde- glutaraldehyde was used to fix brain tissue after the rat perfusion experiments. In this experiment the rat was deeply anesthetized, and then perfused intracardially with a paraformaldehyde- glutaraldehyde solution. The brain was then removed from the rat, placed into a vial containing paraformaldehyde-glutaraldehyde and incubated at least 20 hours. The brain was then removed for further anatomical examination and processed for TEM examination..

Results

Anatomical changes in control and E2 treated female rats of ages 3, 12 and 24 months were assessed in the hippocampus region of female rat brain by TEM. In 3 month old rats, we observed the stable myelin sheath, axon and cytoplasm membranous structure (Fig. 1A).

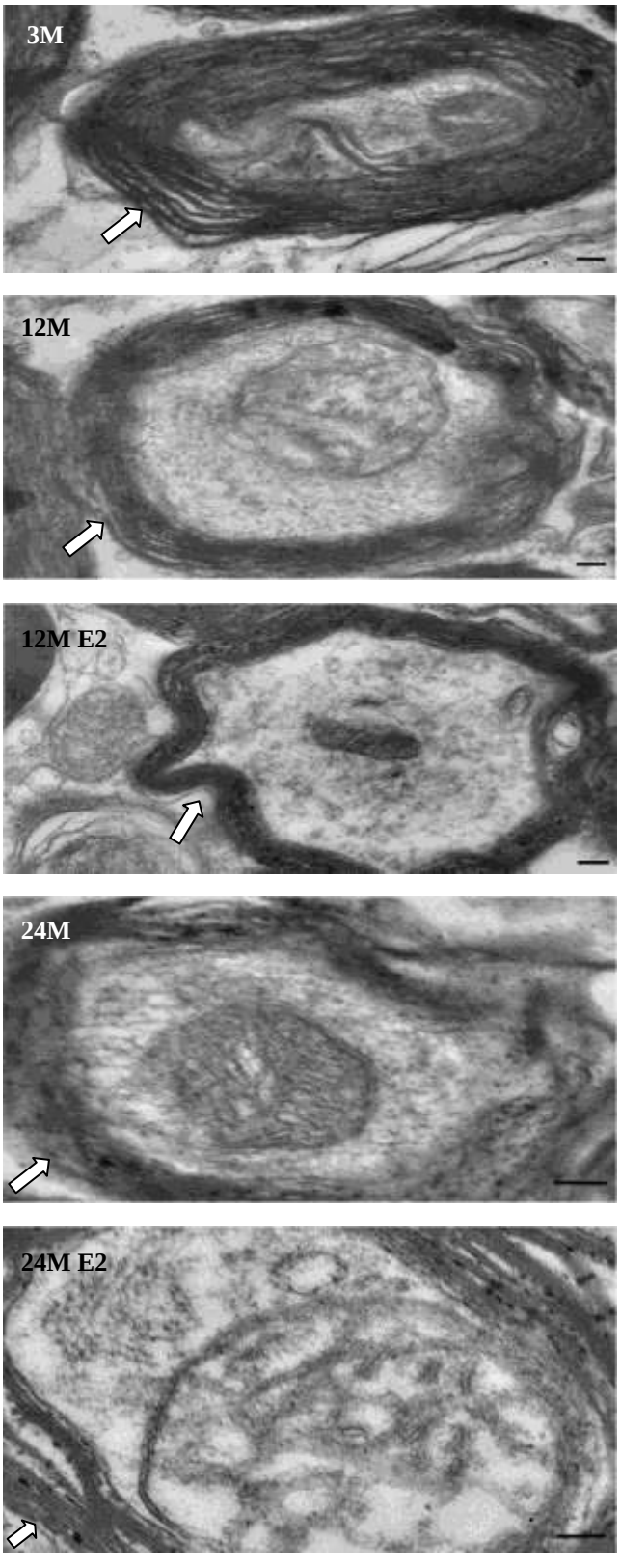

Figure 1 (A-E) Transmission electron micrographs of the rat hippocampus region showing an increase in myelin sheath after E2 treatment (arrow) (Bar = 1μm)

In 12 month old rats we found the age dependent decrease in myelin sheath and changes in axon and cytoplasm membrane (Fig. 1B). However, E2 treated 12

months rat brain show significantly stable myelin sheath, axon and membrane as compared to 12 month control rats (Fig. 1C). Similarly the treatment with E2 in 24 month group showed increase in myelin sheath, changes in axon and stable membranous structure as compared to the 24 month age. In this study, after the treatment with E2 to aging animals, an increase in myelin sheath, axon and cytoplasm membranous stability in hippocampus region was found as compared with 12 and 24 month control rats (Figs. 1 A-E).

Discussion

E2 is synthesized in hippocampus and being present in a high concentration in the hippocampus, hypothalamus, pituitary and amygdale [11]. The aged synapses fundamentally different from the young synapse in its capacity for plasticity, particularly in response to estrogen [23]. In the present study, a quantitative anatomical study was carried out in hippocampus region of female rat brain of different age group, with and without E2 treatment. TEM was used to verify the ultrastructural level. Accurate electron microscope analysis has proved to be very tedious and provides a relatively small sample from which to generalize the result [24, 25]. The anatomical structure study of hippocampus brain region was done by TEM. In this study, progressively with age, decrease in myelin sheath and less stable axon and cytoplasm structure were found. This observation supported by earlier report of Bertoni-Freddari et al.,1993, 2006 and Solmi et al., 1994) [26-28]. Earlier results also showed the changes in the fluidity of membrane and myelin sheath are known to occur during aging [29-31]. Further we studied the anatomical structure of hippocampus region of aging rats in E2 treated rats and found significant increase in myelin sheath and stable cytoplasm membrane in 12 month and 24 months aging rats. Changes were seen in cytoplasm, plasma membrane, myelin axon as well as mitochondrial membrane. From this result we can say that estrogens significantly affect the microstructure of brain regions and state that E2 maintains cytoplasm membrane and myelin axon of aged rats.

This study suggests that significant morphological variations may exist among different neurotransmitter-specific nerve terminals in the brain. E2 interact with neuronal networks at many different levels and affect brain development and aging. However, it is possible that various effects of homogenization may produce some of the morphologic differences. These analyses revealed that fundamental age-related changes in brain and estrogen have important implications when estrogen levels and hippocampal dependent functions decline.

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Conflict of Interest

The authors declare no conflicts of interest.

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