Anti-lipidperoxidative role of exogenous dehydroepiandrosterone (DHEA) administration in normal ageing rat brain

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Effects of exogenous dehydroepiandrosterone (DHEA) administration on the levels of lipid peroxidation products, malondialdehyde (MDA)—a thiobarbituric acid reactive substance (TBARS) and 4-hydroxynonenal (4-HNE) in different brain regions viz. cerebral cortex, hippocampus cerebellum, and brain stem of 12 and 22 months old rats were studied. DHEA treatment significantly depressed TBARS and 4-HNE in all the brain regions studied, in both the age group rats. Interestingly, the magnitude of decrease was higher in the 22 months old rats than that in 12 months old rats. The results suggest that older the animal, better will be the response of exogenous DHEA administration against age-related peroxidative products.

Keywords: Ageing, Anti-lipidperoxidation, Brain, Dehydroepiandrosterone, DHEA, Rat

The decrease in ability to remove deleterious free radicals generated due to variety of oxidation products derived from nucleic acids, sugars, sterols and lipids is believed to increase with increase in age. Of the numerous such products, lipid peroxidation resulting, marker compounds like malondialdehyde (MDA)—a thiobarbituric acid reactive substance (TBARS) and 4-hydroxynonenal (4-HNE)—fluorescence product have been largely demonstrated to increase during ageing process. Inhibition of such cytotoxic product accumulations through pharmacological and dietary interventions has been of a great interest of the gerontologists for the last two decades. Possibility of inhibition of such second toxic messengers through neurosteroidal interventions had emerged decade back and a new role for DHEA as antioxidant had been postulated. Though, DHEA has been widely investigated for its neuroprotective and neurodevelopment effects in both human and animal models and described as youth hormone in humans, but on ageing brain functions, like lipid peroxidation and its products, knowledge related to the in vivo role of exogenous DHEA is still inadequate.

Dehydroepiandrosterone (DHEA)—a precursor of corticosteroid hormones have been shown to remain at higher levels in the brain than other organs particularly in the younger age groups, in both humans and rodents. In addition to adrenal gland it is also synthesized in the brain tissue. Number of studies made so far indicates the beneficial role of DHEA in ameliorating the neurobiological disorders. For example, exposure of DHEA enhanced neural and glial cell survival as compared to controls, protected against excitotoxic effects of NMDA and increased long-term potentiation in the hippocampus. Since, subsequent fall in the levels of DHEA synthesis with age correlate with variety of neurological functions, it has been of a great interest to investigate the possibility of DHEA in inhibiting and reversing age-related parameters. Further, there are reports suggesting the antioxidative properties of DHEA. For example, neuroprotection against oxidative stress induced by hyperglycemia, CuSO₄ and H₂O₂/FeSO₄ and anoxia. It is thus hypothesized that DHEA would have anti-lipidoxidative abilities in the ageing process, particularly in the brain, if administered exogenously.

Since, progressive increase in the levels of lipid peroxidation products viz., MDA or TBARS and 4-HNE in the brain tissue of rat and mice, are considered as marker compounds of the ageing process and correlate with variety of age-associated brain dysfunctions, in the present study the effect of exogenous administration of DHEA has been investigated on the levels of MDA and 4-HNE concentration in the aged rat brain. Brain being a heterogeneous organ, structur-
ally as well as functionally, both the parameters have been measured in different regions of the brain, viz., cerebral cortex, cerebellum, hippocampus and medulla to note the regional selectivity in response of DHEA’s action. As during ageing DHEA levels start declining from 12 months of age onwards, 12 and 22 months old rats have been used for the treatment of DHEA and were defined as early old and late old respectively. Under our animal facility conditions maximum life span of Wistar rats is 24-26 months.

Materials and Methods

Animals—Male Wistar rats of 12 and 22 months age were used. The animals were housed at 22°±2°C under 0800 to 2000 hr light. The rats were provided with food (commercial rat food pellets from Hindustan Lever Ltd, Delhi) and water ad libitum. Each rat was checked for health status by observing various criteria such as tail sores, posture hunch, grooming, nose red rim, red eye rim, tumors, teeth, etc. as reported earlier.

DHEA administration—Experimental animals were administered intraperitoneally (ip) with DHEA (Sigma Chemicals Company, USA) dissolved in dimethylsulphoxide (DMSO) at a dose of 30 mg/kg body wt. daily for one month. Body weight, food and water intake were recorded daily, which did not differ significantly from that of age-matched controls. Rats were assigned to the following four groups as given in Table 1. Group II served as a control for Group I animals and Group IV served as control for Group III animals. Dose of the DHEA was based on the studies of Garcia de Yebenes et al.

Biochemical assays—MDA and 4-HNE were assayed in the whole homogenate from cortex, hippocampus, cerebellum, and medulla of the brains of all the animals (n=5) belonging to each age-group.

After one month the animals were killed by cervical dislocation. After decapitation, the skull was rapidly dissected open and the brains were promptly excised and washed thoroughly with ice cold physiological saline solution. The entire cerebral hemisphere, properly separated from the cerebellum, hippocampus and the medulla was weighed and homogenized in 20 vol of 0.25 M sucrose buffered with 20 mM triethanolamine at pH 7.4.

MDA was estimated in the whole homogenate as per Rehnerona et al. Briefly, sample (0.25 ml) was added to 0.25 ml of 20% trichloroacetic acid and centrifuged for 4 min at 1000 rpm. Thiobarbituric acid (0.5 ml 0.67% in 0.026 M Tris buffer) was added to the supernatant and heated at 100°C for 15-30 min. After cooling in ice for 10 min the absorbance was read at 532 nm using Shimadzu spectrophotometer. Lipid peroxide levels were expressed as n moles malonaldehyde formed/g tissue.

4-HNE was estimated spectrophotometrically according to the method of Esterbauer et al. Briefly 0.5 ml of the sample fraction was mixed with 1.875 ml of chloroform/methanol (1:2 v/v) and centrifuged. To the supernatant, 0.625 ml of chloroform and 0.625 ml of distilled water were added. Sample (1 ml) from the lower chloroform layer was taken out and mixed with 0.1 ml of methanol. The fluorescence intensity of this solution was measured with Shimadzu RF-540 spectrofluorometer at an excitation wavelength of 360 nm and emission wavelength of 430 nm by using quinine sulphate (0.1 µg/ml) in 0.05 M H2SO4 as a standard.

Statistical analysis—The data between DHEA treated and corresponding age-matched control groups were statistically evaluated by using the Student’s t test.

Results and Discussion

Lipid peroxidation product malondialdehyde (MDA)-a thiobarbituric acid reactive substance (TBARS) and the concentration of fluorescence product-4-hydroxynonenal (4-HNE) were measured in the whole homogenate from cerebral cortex, hippocampus, cerebellum and medulla of the brains from 12 and 22 months old treated with DHEA for one month as well as their respective controls.

Figure 1 depicts significant decrease in the levels of MDA in DHEA treated rats of both Group I (P<0.01) and Group III (P<0.001), in all the four different brain regions when compared to their respective controls (Group II and Group IV). Comparing the magnitude of decrease between both the groups, for the level of MDA, it was higher in group III animals than that in Group I animal in all the brain regions, though the regional variation was much prominent in group I animals. Similarly, the magnitude of decrease for 4-
HNE was higher in group III animals than that in Group I animals in all the four regions (Table 2).

Present study is focused mainly on the in vivo anti lipid peroxidative role of exogenous administration of DHEA on the different brain regions of the normal ageing rats. Earlier studies, suggesting antilipidoxida
tive role of DHEA particularly, on the brain tissue are largely made either in vivo or (i) on the experimentally-induced oxidative stressed rats, (ii) animals selected randomly with respect to their body weight and (iii) using very short duration treatment. For example Aregno et al.\textsuperscript{18} showed the inhibitory effect of DHEA on the brain tissue of hypoglycemia induced oxidative stress rats ranging from 200-250 g body weight. Similarly, Boccuzzi et al.\textsuperscript{19} studied its protective effects against copper-induced lipid peroxidation in the rats ranging from 200-250 g body weight. Contrarily to the above, in the present study we used normal ageing rats of 12 and 22 months selected, randomly from the pool of the rats having average life span of 26-28 months and treated with DHEA intraperitoneally for one month, and estimated the levels of lipid peroxidation products viz MDA and 4-HNE, unlike earlier studies, in the four different regions of the brain like cortex, hippocampus, cerebellum and brain stem, hypothesizing that brain is heterogeneous, structurally as well as functionally and each region may respond differentially to DHEA. Data from DHEA pretreated normal ageing rats showed significant decline in the levels of MDA and 4-HNE in all the brain regions studied, irrespective of magnitude of change in individual region. This suggests that DHEA has an ability to improve in vivo the performance of the neurons in ageing brain, unlike, in vitro culture experiments done by earlier worker using different doses for variable durations\textsuperscript{16-18}. As far as regional responses are concerned, variability in the magnitude of change in the levels of MDA and 4-HNE amongst cerebral cortex, hippocampus, cerebellum and brain stem was quite prominent in 12 months old rats. As clear in Table 2 for MDA it varies from minimum 17.31\% in brain stem to maximum 32.57\% in cerebral cortex. Whereas, in 22 months old DHEA pretreated rats the intra regional variability in the magnitude of change was narrowed to minimum 55.69\% in the brain stem and maximum to 59.75\% in the cerebral cortex, compared with their age-matched controls. This shows that initially, at 12 months of age different brain regions respond differently to the exogenous DHEA and with advancing age, this inter regional differences becomes narrow by 22 months of age in rats, which however, suggests that in old age responsiveness of neurons in all the brain regions to exogenous DHEA, in particular, increases with age. Whereas, in 12 months old rats, may be it is the high level of endogenous DHEA which does not allow exogenously administered DHEA to accommodate its binding sites and with age-related decline in the endogenous DHEA level after 12 months, the responsiveness of neurons to exogenous DHEA predominate in 22 months old rats as observed in the present investigation. Though there are reports indicating sim-

![Figure 1](image-url)

Fig.1.—Decrease in the levels of MDA (a) and 4-HNE (b) in different regions of the brain after DHEA treatment. [Cor: cortex, Hippo: hippocampus, CB: cerebellum, BS: brain stem. \(P\) values: *<0.01 and **<0.001 (with reference to their respective controls)]
ply the presence of intracellular as well as membrane-binding sites for DHEA in liver and uterine cervical fibroblasts, but whether such receptors are also present in brain tissue is still unclear. Few studies, for example, Li et al. and Garcia de Yebenes et al. have reported the alteration in the mRNA levels in neuronal tissues after DHEA administration and suggested it as an indicative of DHEA interacts with nuclear sites, but whether such interactions are age-dependent needs extensive investigations to understand the exact role of DHEA particularly in the ageing brain tissue. However, an overall view emerging from the present data indicates that exogenous DHEA is capable of inhibiting the age-related increase in the lipid peroxidation products in the brain tissues. Interestingly, it shows better response in the older age groups against peroxidative products and damages resulting due to age-related increase in lipid peroxidation in the brain.

Short term treatment with lower doses of DHEA has been shown to have protective effects against lipid peroxidation. For example, Aregno et al. used DHEA at a dose of 10, 50 and 100 mg/kg/body wt and suggested the dose of 100 mg/kg/body wt as most potent against lipid peroxidation, while lower doses of 10 and 50 mg/kg/body wt did not show any effect. Similarly, Buccuzi et al. with the same dose showed the antioxidative effects of the drug. Both sacrificed the rats after 3 hr and 17 hr of DHEA pretreatment respectively. In the present experiment, DHEA was given for one month and significant decrease was recorded in both the lipid peroxidation products even at still lower dose of 30 mg/kg body wt which is in accordance to the earlier study which suggested that greatest number of DHEA’s actions on nervous system require doses below 60 mg/kg body wt. Even the dose of 50 mg/kg was shown higher enough for chronic treatment, which resulted in increased mortality rate after 15 days. The dose of 30 mg/kg/body wt did not affect mortality rate which is further in accordance to the observations of Wan et al. Therefore, in view of earlier reports as well as present observations, in conclusion it could safely be suggested that in comparison to early old (12 months) animals, in aged, lower doses of DHEA for longer duration could be more beneficial rather than higher doses for short durations in order to achieve better protection against age-related peroxidative damages in the nervous tissue.

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**References**