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Exogenous administration of dehydroepiendrosterone attenuates loss of superoxide dismuatse activity in the brain of old rats

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The influence of exogenously administered dehyroepiendrosterone (DHEA) on the activity of superoxide dismutase (SOD) was investigated in the mitochondrial and cytosolic fractions from cerebral cortex, cerebellum, hippocampus and medulla regions of the brains of 12- and 22-months old rats. DHEA was administered daily at the dose of 30 mg/kg/body wt, intraperitonially (i.p) in both age groups of rats for 1 month. Results showed that SOD activity was significantly higher in the mitochondrial fraction than in the cytosolic fraction, in DHEA-treated animals in both age groups. This indicated that exogenous DHEA affected mitochondrial SOD more than the cytosolic SOD. In terms of percent increase, 22 months-old animals showed significant increase in the SOD activity in both the fractions of all the four brain regions than in the 12 months old DHEA-treated animals. This showed that exogenous DHEA provided more protection to the SOD in ageing brain of older rats (22 months) than the younger (12 month) ones. The study suggests that exogenous DHEA is more beneficial at old age in terms of neuroprotection against oxidative stress-mediated brain dysfunctions and may protect age-related alterations in cognitive functions like learning and memory.

Keywords: Dehydroepiendrosterone, Superoxide dismutase, Brain, Aging

Dehydroepiandrosterone (DHEA), a precursor of corticosteroid hormones, though synthesized in the adrenal cortex, its level in brain is higher than in other organs, particularly in the younger age groups, both in humans and rodents. The subsequent fall in the DHEA levels with age correlates with variety of neurological disorders, as it may leave the brain unprotected against neurotoxic challenges[1]. Enhanced neural and glial cell survival is reported in the hippocampus after the exposure of DHEA. It also ameliorates the neurobiological alterations known to occur with age[2-4].

DHEA provides neuroprotection against oxidative stress induced by hyperglycemia[5], CCl_4[6], $CuSO4$[7], $H_2O_2/FeSO_4$[8], anoxia[9], streptozotocin[10] and in diabetic rats[11]. Recently, the mechanism of protective effect of DHEA on oxidative stress, excitotoxicity and apoptosis has been reported[12]. DHEA also modulates the physiology and pathology of the brain on many aspects such as learning and memory, synaptic transmission, neurodegenerative diseases (especially Alzheimer's disease), emotion, stress and menstrual cycle-linked disorders[13]. In castrated mice, high levels of DHEA in brain have shown an anti-anxiety-like and an anti-depressive-like effect and low levels in frontal cortex results in vulnerability to depression and/or anxiety[14]. Very few reports, however, are available on the effects of DHEA on normal ageing processes in the brain.

Superoxide dismuatse (SOD) is involved in cellular detoxification against superoxide anions. High lipid content and oxygen consumption make the brain vulnerable for the generation of superoxide radicals. Since brain is poor in cellular defence due to lower antioxidant enzyme activities including SOD, reactive oxygen species (ROS) generated by oxidative stress is believed to be one of the primary factors contributing to brain ageing. Though the exact mechanism underlying the process of ageing is not fully understood, evidences suggest mitochondria is a major site of superoxide anion formation[15-19].

There are reports showing age-related loss of SOD in the rat brain[20,21]. Whether such changes could be inhibited or reversed through pharmacological interventions has been an area of great interest. *In vitro* studies have shown enhanced survival of neurons in culture after DHEA treatment by way of increased activities of SOD and GPx[22]. Though antioxidative role of DHEA against variety of induced oxidative stress has been widely documented[5-11], but

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whether exogenous administration of DHEA plays any beneficial role on the SOD activity in ageing brain is hardly been studied.

Mitochondria, being site of cellular respiration are greatly involved in free radical generation leading to the oxidative stress[16,17,19]. Since mitochondria have shown affinity for DHEA in the developing rats[23] and DHEA affects mitochondrial dehydrogenases activities in both young and old age-groups[24], in the present study, we have measured the activity of SOD in both cytosolic and mitochondrial fractions in two old age groups of rats (aged 12 and 22 months) in different brain regions and have studied the changes in SOD with DHEA administration in both age groups.

Materials and Methods

Animals

Male Wistar rats of 12 and 22 months age were used. The animals were housed at 22 $\pm$2°C under 0800 to 2000 h light and provided with food, commercial rat food pellets (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, and tumors etc[25]. The experimentation protocol was approved by the Institutional Animal Ethical Committee (IAEC), JNU, New Delhi.

DHEA administration

Experimental animals were administered DHEA (Sigma Chemicals Co., USA), dissolved in dimethylsulphoxide (DMSO) at a dose of 30 mg/kg body wt daily intraperitoneally (i.p) for 1 month according to protocol described previously[26-28]. Body weight, food and water intake were recorded daily, which did not differ significantly from that of age-matched controls. Rats were divided into the four different groups. Group I: 12 months old rats (n = 6) were treated with DHEA for 1 month. Group II: 12 months old rats (n = 6) were treated with DMSO (vehicle) for 1 month and served as controls for group I. Group III: 22 month old rats (n = 6) were treated with DHEA for 1 month and Group IV animals of 22 month old (n = 6) were given DMSO for 1 month and served as controls for group III.

Animals were sacrificed by cervical dislocation, the skull was rapidly dissected open and the brains were promptly excised and washed thoroughly with ice-cold physiological saline solution. The cerebral hemisphere, cerebellum, hippocampus and medulla were separated out, weighed and homogenized in sucrose isotonic buffer (0.32 *M* sucrose, 12.5 m*M* Tris and 1 m*M* EDTA, *p*H 7.4, dilution of 1:19) and processed by differential centrifugation for separation of subcelluar fractions[29,30]. The crude homogenates of brain regions were initially centrifuged at 1000 rpm for 10 min and the supernatants obtained were again centrifuged at 25,000 rpm for 30 min to separate cytosol as well as the pellet containing mitochondrial fraction. The pellet was washed two-three times by homogenizing in same buffer (*p*H 7.4) and centrifuged again at 25,000 for 30 min. Both fractions were used for assays of SOD. All the procedures were carried out at 0-4°C

Assay of SOD

The SOD activity in the pellet and cytosolic fractions was determined as described previously[40]. The final volume (500 µl) of assay mixture contained: 50 µl 10 m*M* EDTA + 25 µl 2.5 m*M* pyrogallol (in 10 m*M* HCl), tissue sample (5-10 µl) and 425 µl of 50 m*M* tris HCl (*p*H 8.2) was added in the 750 µl quartz cuvette. The change in absorbance was measured at 420 nm for 10 min using a UV-260 A Spectrophotometer. One unit of enzyme was defined as the amount of SOD required that produced 50% inhibition of auto-oxidation of pyrogallol and specific activity of SOD was expressed as Unit/min/mg protein

Protein content in both cytosolic and pellet fractions was estimated with Lowry's method using bovine serum albumin (BSA) as the standard[32].

Statistical analysis

The statistical evaluations between DHEA-treated and corresponding age-matched control group was calculated using student's 't' test.

Results and Discussion

The changes in SOD activity in cytosol and pellet (synaptosomal + mitochondrial) fractions in different brain regions of ageing animals and the effect of DHEA are shown in Fig. 1. The SOD activity decreased significantly in 22 months old rats, compared to the 12 months animals in all the four brain regions. This indicated age-related decrease in the SOD activity which was in accordance with the earlier report[20], wherein such age-matched decline was observed in the cortex and cerebellum in rats from 12 months, up to 21 months of age in rats. Age-related decrease in SOD activity was also observed in

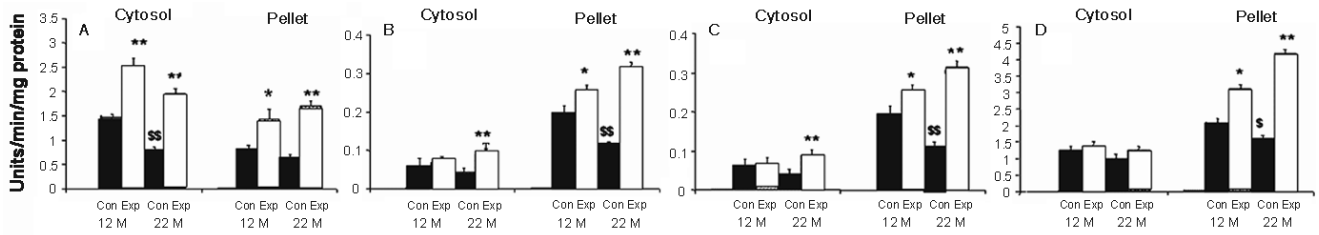

Fig. 1—SOD activity in (A) cerebral cortex (B) hippocampus (C) cerebellum (D) medulla regions of brain in 12 and 22 months old control and DHEA-treated rats [Decrease in SOD activity from 12 to 22 month of age, significant increase after DHEA treatment, particularly in pellet fraction in both age groups compared to age-matched controls is evident. $^{\$\$}p<0.01,^{\$}p<0.05$, (**p<0.01;*p<0.05]

the homogenates from cerebrum, hypothalamus, hippocampus, cerebellum, brain stem, spinal cord[33] and in brain tissues of female rat[34]. In the present study, we estimated the activity in both cytosolic and pellet fractions. Our findings for the first time substantiated the effect of DHEA in the ageing brain and were in agreement with earlier study[25], which demonstrated that age-related decline in SOD activity in brain homogenates was mainly because of significant changes in the mitochondrial SOD, as compared to the cytosolic SOD.

After DHEA treatment, increase of SOD activity was significantly higher in 22 month old than in the 12 months old DHEA-treated rats in both cytosolic and pellet fractions. Fig. 1 also shows that in DHEA-treated rats, SOD activity was maximum in the cortex in both fractions. No significant increase was observed in activity in the cerebellum, hippocampus and medulla in the cytosolic fraction, except in the pellet fraction. Table 1 shows the percentage increase in SOD activity in the pellet and cytosolic fractions of the four brain regions after DHEA treatment. Older age group showed more percent increase than younger ones. Also, there was a variation in the percent change in activity among the brain regions, between cytosolic and pellet fractions. Cerebellum showed highest increase after DHEA treatment in the older age groups in both pellet and cytosolic fractions, whereas, in medulla percent increase was minimum in both the fractions.

In human brain, increasing mitochondrial deletions have shown regional variability with age[35]. Thus, in the present study, DHEA was administered for 1 month in two different age groups of rats (12 and 22 months old) and SOD activity in four different brain regions was estimated in the cytosolic and pellet fractions of different brain regions. Increased SOD activity in all the brain regions on exogenous administration of DHEA might be responsible for

Table 1—Percent increase in SOD activity in the cytosolic and pellet fractions after DHEA treatment in 12 and 22 months rat brain regions

Fractions	Brain regions	Percent increase	
		Group I (12 M)	Group II (22 M)
Cytosol	Cortex	40	63
	Cerebellum	16	66
	Hippocampus	16	56
	Medulla	15	20
Pellet	Cortex	48	63
	Cerebellum	26	65
	Hippocampus	30	66
	Medulla	32	63

making the tissue resistant to the free radical generation. Our results were in agreement with earlier reports[5,7,9,36-39], which suggested that DHEA makes the tissue more resistant to lipid peroxidation and behaves almost parallel to α-tocopherol, a potent free radical scavenger, in terms of thiobarbuteric acid production.

Induction of hepatic mitochondrial glycro-phosphate dehydrogenase has been reported in old rats by DHEA[40]. Since exogenous DHEA has been reported to accelerate the maturation of cerebral mitochondria[23], increased SOD activity in the pellet fraction of both the regions from 12 and 22 months old DHEA-treated rats signifies that DHEA has affinity for mitochondria to combat with the age-related free radical-mediated oxidative stress. An increase in the total phospholipids and cholesterol in the brain mitochondria is also reported after DHEA treatment and these changes might compliment the electron transport chain components[41]. Furthermore, evidences suggest that DHEA administration affects mitochondrial respiration and thereby leading to weight loss[42]. Recently, it has been reported that treatment with DHEA significantly stimulates

oxidative energy metabolism in rat liver and brain mitochondria[43]. Therefore, present findings that exogenously administered DHEA improved SOD activity in the brain, particularly in the mitochondria substantiate the knowledge of DHEA's antioxidative capabilities in ageing brain. Because 22 months old DHEA-treated rats have shown higher increase in SOD activity than 12 months old rats in both the fractions, it may be concluded that exogenous DHEA is more effective in older age or at the onset of ageing process than in younger ones. It could protect brain against age-related increase in oxidative stress-induced neuronal toxicity[44] involving the release of glutamate[45] and age-related brain dysfunctions including cognitive functions.

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