Chapter 6

Treatment with dehydroepiandrosterone (DHEA) stimulates oxidative energy metabolism in the cerebral mitochondria- A comparative study of effects in old and young adult rats

Introduction

Results of the previous chapter revealed that DHEA treatment influenced mitochondrial development and maturation positively. But we also found that stimulation of respiratory rates was age dependent. It is of higher magnitude in case of developing rats i.e. 3 week and 5 week than that of young adult rats (chapter 4 and 5). This may be because of a characteristic age-related pattern of DHEA. The concentration of DHEA in the serum is low in infancy, starts increasing in adolescence, and reaches a peak in adult life around 20-30 year. The level starts declining thereafter around the age of 35-40 years and in the elderly the levels decrease substantially (Deshmukh and Patel, 1980; Hinson and Raven, 1999; Parker, 1999). Based on this characteristic profile, DHEA is considered to be the **YOUTH HORMONE** (Celec and Starka, 2003; Hinson and Raven, 1999).

Interestingly, DHEA and its sulfated conjugate DHEA-S, and pregnenolone are known to be synthesized by the brain and are considered as neurosteroids (Racchi, Balduzzi and Corsini, 2003). The concentration of DHEA is very low (ng range/g tissue) with the highest concentration being seen in the anterior pituitary (Corpechot et al., 1981; Ren and Hou, 2005; Vallee M et al., 2000; Weill-Engerer et al., 2002). The concentration of DHEA-S is about 10 time higher (Corpechot et al., 1981). An age-dependent decline in the content of DHEA-S in the brain has been reported (Kazihnitkova et al., 2004). Presences of DHEA and DHEA-S in human brain and a decrease in their contents in Alzheimer's disease and dementia have been reported (Weill-Engerer et al., 2002). There are reports to indicate that the exogenous supplementation with DHEA helps to improve memory and behaviour in elderly population (Buvat, 2003). In rats fed diet supplemented with DHEA, significant stimulation of mitochondrial function and proliferation of mitochondria have been reported in liver (Bellei et al., 1992; Min Kyung et al., 1989).

Of the total oxygen consumption by the body about 20% oxygen is utilized in the brain (Erecinska and Silver, 2001) which is consistent with the fact that the

electrophysiological functions of the brain are known to be energy dependent (Astrup, Sorensen and Sorensen, 1981; Attwell and Laughlin, 2001).

Therefore, it is of interest to understand if treatment with exogenous DHEA has any beneficial effect on improving memory and behaviour in elderly population due to its ability of improving cerebral mitochondrial energy transduction. We tested this possibility by treating old male rats with DHEA.

Materials and methods

Chemicals

Chemicals used were same as described in chapter 2.

Animals and treatment with DHEA

Male young adult (8-10 week old) and old (18-24 month old) albino rats of Charles-Foster strain were used. At the start of experiment the body weight of young adult rats was in the range of 235-240 g while that of old rats was in the range of 358-375 g. The animals were injected subcutaneously (s.c.) with 0.2 mg or 1.0 mg DHEA per kg body weight for seven consecutive days. The number of animals in the different groups ranged from 12 to 20. DHEA suspension was prepared fresh in saline prior to use. The controls received equivalent volume of saline vehicle. The animals were sacrificed on the 8th day for isolation of mitochondria.

Other methods are described in chapter 2.

All results are given as mean ± SEM.

Statistical evaluation of the data was by Students' t-test.

Results

Treatment with 1.0 mg DHEA resulted in 17% increase in body weight of young adult rats without having any effect on the brain weight. By contrast, DHEA treatment had no appreciable effect on body weight in the old rats. However, treatment with 1.0 mg DHEA resulted in a small but significant increase (4%) in brain weight (data not given). Possibly, the increased respiratory metabolism (detailed below) may have induced the increase in the brain weight.

Oxidative phosphorylation

General

The effects of DHEA treatment on oxidative energy metabolism are summarized in Tables 1-4. In untreated old rats the state 3 respiration rates and ADP-phosphorylation rates were about 16-38% lower compared to those in the corresponding young adult rats for the different substrate used. The state 4 respiration rate increased with pyruvate + malate with the opposite seen for succinate. Consequently, the respiratory control ratios were low for all the substrate which is indicative of mitochondrial fragility; fragility of cerebral mitochondria in old mice has been reported (Navarro et al., 2005). Our observations are consistent with those reported by other researchers (Cocco et al., 2005; Corpechot et al., 1981; Ferrandiz et al., 1994; Navarro et al., 2005). The results thus emphasize decreased energy metabolising potential of brain with aging.

Effects of DHEA treatment

DHEA treatment of young animals brought about significant stimulation of respiratory activities with glutamate, pyruvate + malate, succinate and ascorbate + TMPD in a dosedependent manner. These changes were also reflected in terms of corresponding increase in the ADP-phosphorylation rates (Tables 1-4). The state 4 respiration rates also showed parallel increase.

DHEA treatment also resulted in increase in the contents of cytochrome aa_3 and b in a dose-dependent manner. Interestingly the content of cytochrome $c+c_1$ was unchanged (Table 5).

Likewise, the ATPase activities also increased after DHEA treatment (Table 6).

A similar picture was obtained for glutamate dehydrogenase (GDH), mitochondrial malate dehydrogenase (MDH) and succinate DCIP reductase (SDR) with the effect being more pronounced with 0.2 mg DHEA. The cytosolic MDH was not affected (Fig. 1).

Table 1: Effect of DHEA treatment on oxidative phosphorylation in rat brain mitochondria using glutamate as the substrate

Age group	Treatment	ADP/O ratio	Respiration rate (nmole O_2/ min/mg protein)		Respiratory Control Ratio	ADP phosphorylation rate (nmole /min/ mg protein)
			+ ADP	-ADP		
Young Adult	Untreated (12)	3.09 ± 0.10	18.23 ± 0.79	4.56 ± 0.21	4.04 ± 0.15	111.80 ± 4.68
	0.2 mg DHEA (12)	3.03 ± 0.09	29.73 ± 2.01[a]	7.88 ± 0.36[a]	3.75 ± 0.14	180.00 ± 10.17[a]
	1.0 mg DHEA (12)	3.01 ± 0.12	33.38 ± 0.75[a]	18.53 ± 0.67[a]	1.88 ± 0.08	193.70 ± 6.40[a]
Old	Untreated (20)	3.03 ± 0.17	13.06 ± 0.63*	4.81 ± 0.24	2.88 ± 0.13*	76.79 ± 4.54*
	0.2 mg DHEA (12)	3.01 ± 0.13	23.31 ± 1.78[a]	8.71 ± 0.61[a]	2.74 ± 0.13	139.50 ± 11.10[a]
	1.0 mg DHEA (12)	3.00 ± 0.12	20.70 ± 0.45[a]	8.38 ± 0.41[a]	2.51 ± 0.11	124.00 ± 5.38[a]

Experimental details are as given in the text. Results are given as mean ± S.E.M. of the number of observations indicated in the parentheses.

a $p < 0.001$ compared with the corresponding untreated group.

* $p < 0.001$ compared with the untreated young adult group.

Table 2: Effect of DHEA treatment on oxidative phosphorylation in rat brain mitochondria using pyruvate + malate as the substrate

Age group	Treatment	ADP/O ratio	Respiration rate (nmole O_2/ min/mg protein)		Respiratory Control Ratio	ADP phosphorylation rate (nmole /min/ mg protein)
			+ ADP	-ADP		
Young Adult	Untreated (12)	3.10 ± 0.10	21.49 ± 1.14	4.71 ± 0.27	4.64 ± 0.23	132.30 ± 4.37
	0.2 mg DHEA (12)	3.13 ± 0.08	22.96 ± 0.76	7.92 ± 0.39[c]	2.97 ± 0.16	143.20 ± 4.87
	1.0 mg DHEA (12)	3.13 ± 0.10	26.92 ± 0.92[b]	8.01 ± 0.36[c]	3.34 ± 0.28	169.10 ± 9.05[b]
Old	Untreated (16)	3.09 ± 0.14	18.00 ± 0.72*	7.28 ± 0.40***	2.61 ± 0.17***	109.90 ± 5.43**
	0.2 mg DHEA (12)	3.14 ± 0.17	20.35 ± 1.00	8.96 ± 0.25[b]	2.29 ± 0.12	126.00 ± 6.61
	1.0 mg DHEA (9)	3.12 ± 0.13	23.40 ± 1.03[c]	8.97 ± 0.40[a]	2.97 ± 0.17	145.80 ± 8.07[b]

Experimental details are as given in the text. Results are given as mean ± S.E.M. of the number of observations indicated in the parentheses.
a $p < 0.01$, b $p < 0.002$ and c $p < 0.001$ compared with the corresponding untreated group.
* $p < 0.02$, ** $p < 0.01$ and *** $p < 0.001$ compared with the untreated young adult group.

Table 3: Effect of DHEA treatment on oxidative phosphorylation in rat brain mitochondria using succinate as the substrate

Age group	Treatment	ADP/O ratio	Respiration rate (nmole O_2/ min/mg protein)		Respiratory Control Ratio	ADP phosphorylation rate (nmole /min/ mg protein)
			+ ADP	-ADP		
Young Adult	Untreated (12)	2.07 ± 0.06	25.17 ± 1.42	14.18 ± 1.05	1.87 ± 0.16	103.60 ± 5.96
	0.2 mg DHEA (12)	2.17 ± 0.10	33.90 ± 0.95^{e}	18.96 ± 0.88^{d}	1.83 ± 0.09	140.00 ± 7.13^{e}
	1.0 mg DHEA (12)	2.10 ± 0.07	28.60 ± 0.99	17.10 ± 0.67^{a}	1.67 ± 0.05	118.90 ± 3.44^{a}
Old	Untreated (16)	2.10 ± 0.15	15.65 ± 0.75***	10.78 ± 0.77**	1.51 ± 0.07*	64.44 ± 4.63***
	0.2 mg DHEA (11)	2.09 ± 0.16	22.07 ± 1.50^{e}	14.96 ± 1.23^{c}	1.53 ± 0.12	91.62 ± 8.39^{b}
	1.0 mg DHEA (8)	2.07 ± 0.13	27.95 ± 1.20^{e}	21.60 ± 1.65^{e}	1.32 ± 0.07	113.20 ± 9.80^{e}

Experimental details are as given in the text. Results are given as mean ± S.E.M. of the number of observations indicated in the parentheses.
a $p < 0.05$, b $p < 0.02$, c $p < 0.01$, d $p < 0.002$ and e $p < 0.001$ compared with the corresponding untreated group.
* $p < 0.05$, ** $p < 0.02$ and *** $p < 0.001$ compared with the untreated young adult group.

Table 4: Effect of DHEA treatment on oxidative phosphorylation in rat brain mitochondria using ascorbate + TMPD as the substrate

Age group	Treatment	ADP/O ratio	Respiration rate (nmole O_2/ min/mg protein)		Respiratory Control Ratio	ADP phosphorylation rate (nmole /min/ mg protein)
			+ ADP	-ADP		
Young Adult	Untreated (12)	0.70 ± 0.03	22.99 ± 1.81	13.92 ± 1.12	1.66 ± 0.04	31.82 ± 2.35
	0.2 mg DHEA (12)	0.72 ± 0.03	28.98 ± 1.76[a]	19.90 ± 1.23[a]	1.46 ± 0.02	41.75 ± 3.26[a]
	1.0 mg DHEA (12)	0.70 ± 0.03	31.44 ± 0.81[e]	19.73 ± 0.90[e]	1.61 ± 0.05	44.32 ± 2.38[d]
Old	Untreated (20)	0.72 ± 0.04	17.43 ± 1.04*	12.32 ± 0.64	1.41 ± 0.03**	24.03 ± 1.56**
	0.2 mg DHEA (16)	0.75 ± 0.05	22.18 ± 1.25[b]	16.83 ± 0.85[e]	1.32 ± 0.04	33.21 ± 2.61[c]
	1.0 mg DHEA (12)	0.73 ± 0.04	22.03 ± 1.11[c]	17.57 ± 0.69[e]	1.25 ± 0.04	32.85 ± 1.38[e]

Experimental details are as given in the text. Results are given as mean ± S.E.M. of the number of observations indicated in the parentheses.
a $p < 0.05$, b $p < 0.02$, c $p < 0.01$, d $p < 0.002$ and e $p < 0.001$ compared with the corresponding untreated group.
* $p < 0.05$ and ** $p < 0.02$compared with the untreated young adult group.

Table 5: Effect of DHEA treatment on the cytochrome content of rat brain mitochondria

Age group	Treatment	Cytochrome content (pmol/mg protein)		
		aa_3	b	$c+c_1$
Young Adult	Untreated (8)	154.6 ± 8.46	176.2 ± 10.21	224.3 ± 14.80
	0.2 mg DHEA (8)	186.3 ± 3.87[b]	193.0 ± 7.64	203.6 ± 9.49
	1.0 mg DHEA (8)	267.4 ± 7.16[c]	353.9 ± 9.77[c]	198.8 ± 12.91
Old	Untreated (12)	96.5 ± 5.39*	185.3 ± 8.51	320.8 ± 16.88*
	0.2 mg DHEA (12)	143.8 ± 6.15[c]	216.5 ± 7.72[a]	304.9 ± 13.02
	1.0 mg DHEA (12)	213.3 ± 6.56[c]	268.0 ± 6.10[c]	247.3 ± 18.05[c]

Experimental details are as given in the text. Results are given as mean ± S.E.M. of the number of observations indicated in the parentheses.
a $p < 0.02$, b $p < 0.01$ and c $p < 0.001$ compared with the corresponding untreated group.
* $p < 0.001$ compared with the untreated young adult group.

Table 6: Effect of DHEA treatment on ATPase activity in rat brain mitochondria

Age group	Treatment	Activity (µmol Pi liberated/h/mg protein)			
		Basal	$+Mg^{2+}$	+DNP	$+Mg^{2+}$+DNP
Young Adult	Untreated (8)	0.36 ± 0.02	6.03 ± 0.21	0.49 ± 0.03	6.86 ± 0.14
	0.2 mg DHEA (8)	0.81 ± 0.05[c]	8.34 ± 0.26[c]	0.89 ± 0.05[c]	8.66 ± 0.21[c]
	1.0 mg DHEA (8)	0.73 ± 0.05[c]	7.98 ± 0.31[c]	0.76 ± 0.05[c]	8.98 ± 0.19[c]
Old	Untreated (12)	0.35 ± 0.02	3.45 ± 0.25*	0.53 ± 0.03	4.08 ± 0.24*
	0.2 mg DHEA (12)	0.35 ± 0.02	4.48 ± 0.19[b]	0.46 ± 0.03	4.74 ± 0.12[a]
	1.0 mg DHEA (12)	0.44 ± 0.02[b]	3.33 ± 0.14	0.47 ± 0.02	3.88 ± 0.16

Experimental details are as given in the text. Results are given as mean ± S.E.M. of the number of observations indicated in the parentheses.
a $p < 0.05$, b $p < 0.01$ and c $p < 0.001$ compared with the corresponding untreated group.
* $p < 0.001$ compared with the untreated young adult group.

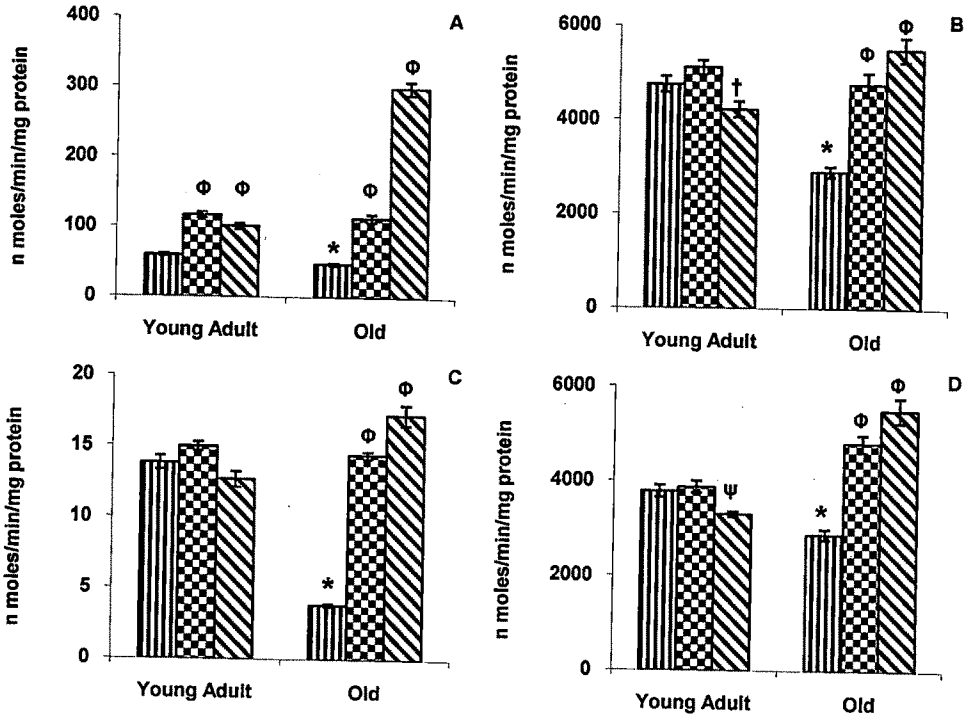

Fig. 1. Effect of DHEA treatment on mitochondrial and cytosolic dehydrogenases activities in rat brain. The results are given as mean ± S.E.M. of 12 and 20 independent observations for young adult and old animals, respectively. (A) Glutamate dehydrogenase; (B) malate dehydrogenase (Mitochondrial); (C) succinate DCIP reductase and (D) malate dehydrogenase (cytosolic);

▥ Untreated; ▩ 0.2 mg DHEA and ▨ 1.0 mg DHEA. † $p < 0.05$; ψ $p < 0.002$ and **Φ** $p < 0.001$ compared with the corresponding untreated group. *$p < 0.001$ compared with the untreated young adult group.

Discussion

Present investigations were undertaken to find out if treatment with exogenous DHEA would accelerate the respiration rates of cerebral mitochondria in old rats. The result showed that in the old rats, treatment with DHEA stimulated the state 3 respiration rates and brought them to the levels comparable to untreated young adults (Tables 1-4). Parallel increase in ADP-phosphorylation rates and state 4 respiration rates were also evident (Tables 1-4). The old rats were characterized by significant decrease in cytochrome aa3 content (Table 5) which agrees well with the low respiration rate that we report here. Decreased complex IV activity in cerebral mitochondria from old mice has been reported (Haripriya et al., 2004). The content of cytochrome b was unchanged but that of c + c_1 increased substantially which could be a compensatory mechanism. Increased transcription of cytochrome c gene in old mice has been reported by other researchers (Manczak et al., 2005). Treatment with DHEA resulted in increasing the contents of cytochrome aa3 and b in a dose-dependent manner. However, higher dose (1.0 mg DHEA) had adverse effect on the content of cytochrome c + c_1 (Table 5). The ATPase activities were generally low in old animals and DHEA treatment had only marginal effect (Table 6). However, following treatment with DHEA the levels of GDH, mitochondrial MDH, and SDR increased substantially reaching values close to or higher than the young adult controls in a dose-dependent manner. A similar trend was evident even for cytosolic MDH activity (Fig. 1).

It has been reported that in the old animals initially there is up-regulation of the genes encoding peptides in complex I, III, IV and V of the respiratory chain which is followed by down-regulation at later stage (Manczak et al., 2005). Our results on the respiratory activity in the cerebral mitochondria from the old rats are consistent with the latter observation. The results of the present study point out that DHEA treatment was effective in enhancing the respiratory potential of cerebral mitochondrial in old as well as young adult rats. The results also show that DHEA treatment was able to restore the respiratory parameters in old animals to values comparable to the untreated young adults. Interestingly, the increase in the respiratory parameters was of higher magnitude in young adults.

In this connection it may be mentioned that in the elderly population the plasma levels of DHEA decrease to about 10% of adult values at the age of around 70 years (Hinson and Raven, 1999; Milgrom, 1990). Considering the life-span of rat as 3 years, the old animals (18-24 month old) in our studies may be equated with old humans of around 62-65 year age. If a similar situation prevails in the rats, then presumably the plasma DHEA levels in the old rats may be expected to be very low compared to the young adults. Age dependent decrease in the content of DHEA in the human and rat brain have also been reported (Kazihnitkova et al., 2004; Weill-Engerer et al., 2002). Therefore, it is possible that even after treating with exogenous DHEA it is unlikely that the adult plasma and/or brain levels of DHEA will be reached in the old animals. This could be one of the possible reasons for lower magnitude of stimulation in old animals. It is also important to note that higher dose of DHEA had no extra beneficial effects in young adults and occasionally showed adverse effects. These observations therefore cautions that dose of exogenous DHEA should be adjusted judiciously.

It is clear from the data presented that GDH and cytosolic as well as mitochondrial MDH activities increased after treatment with DHEA. Additionally, under these treatment conditions the contents of cytochromes aa_3 and b also increased. These changes were accompanied by increase in the ATPase activity (Fig. 1, Tables 5 and 6). The dehydrogenases are known to be nuclear gene products whereas crucial polypeptides of cytochrome oxidase, i.e. cytochromes aa_3 and of cytochrome b and ATPase are known to be coded by mitochondrial DNA (Poyton and Mc Ewen, 1996). It may hence be suggested that DHEA treatment enhanced the respiratory activity of mitochondria by selective activation of specific nuclear and mitochondrial genes. The most interesting part was that the contents of cytochromes $c + c_1$ decreased in the old animals after treatment with 1 mg DHEA, bringing the value close to control young adult level. This may suggest that DHEA has a negative regulatory role in cytochromes $c + c_1$ synthesis. Higher concentrations of DHEA occasionally showed less beneficial effects. It may hence be suggested that intricate age-dependent mechanisms may regulate DHEA action.

The possibility of non-genomic effects seems unlikely for two reasons. Firstly the treatment with DHEA was for a longer duration, i.e. for 1 week. Secondly, non-genomic membrane effects of steroids are evident within minutes (Duval, Durant and Homo-Delarche, 1983). Besides, it has been reported that incubation of brain mitochondria with DHEA under in vitro conditions inhibited all respiratory parameters (Morin et al., 2002). It is well recognized that the concentrations of DHEA-S are several times higher than those of DHEA in all brain regions (Ren and Hou, 2005). DHEA-S is the precursor for cerebral DHEA which is metabolized to 7α hydroxy DHEA and δ5 androstene 3β, 17β diol (Steckelbroeck et al., 2002; Weill-Engerer et al., 2003); the former is considered to be active metabolite (Steckelbroeck et al., 2002; Weill-Engerer et al., 2003). The low concentrations of DHEA in the brain may be attributed to its rapid conversion to 7α hydroxy DHEA and δ5 androstene 3β, 17β diol. It is possible that following treatment with exogenous DHEA there could be increased conversion to the active metabolites, which is responsible for the observed effects in terms of enhanced respiratory activity in the cerebral mitochondria. However, this possibility needs to be verified by more direct experiments using 7α hydroxy DHEA.

In conclusion our results show that treatment with DHEA can improve the respiratory parameters of cerebral mitochondria in old rats bringing them close to young adult levels. Since the electrophysiological function of the brain is known to be energy dependent (Astrup, Sorensen and Sorensen 1981; Attwell and Laughlin, 2001) the enhanced respiratory function can help in improving memory and behavioral pattern in elderly. This data leads to a suggestion that DHEA may also positively affect liver mitochondria in old rats. This is studied in next chapter.

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